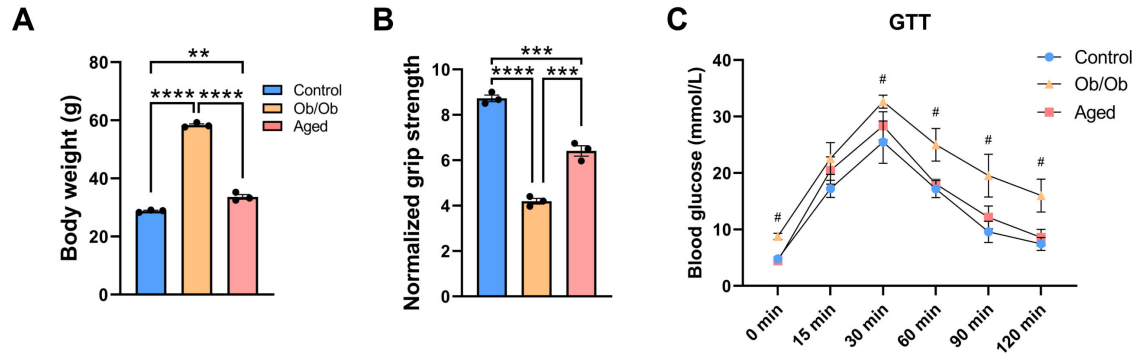
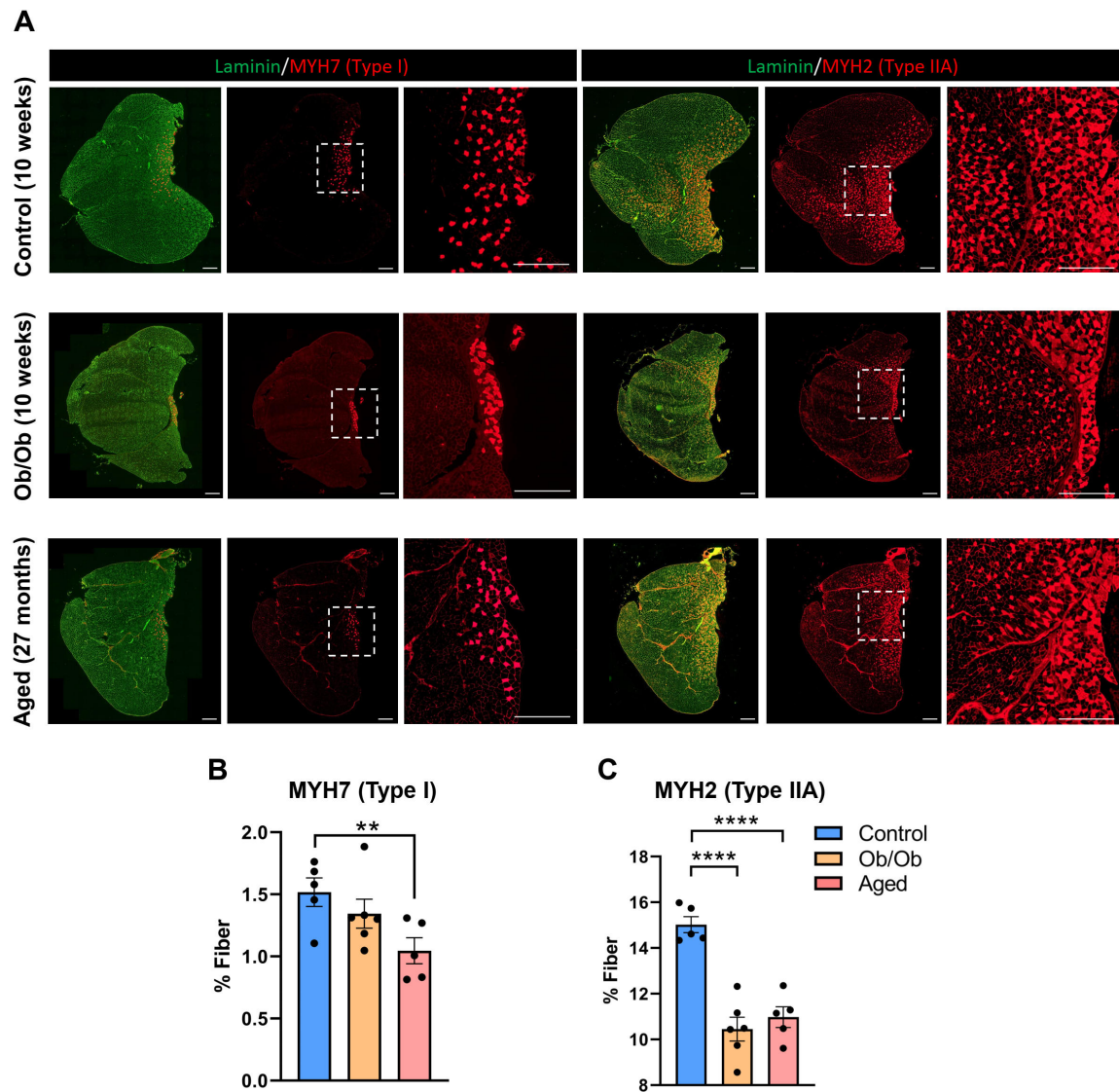
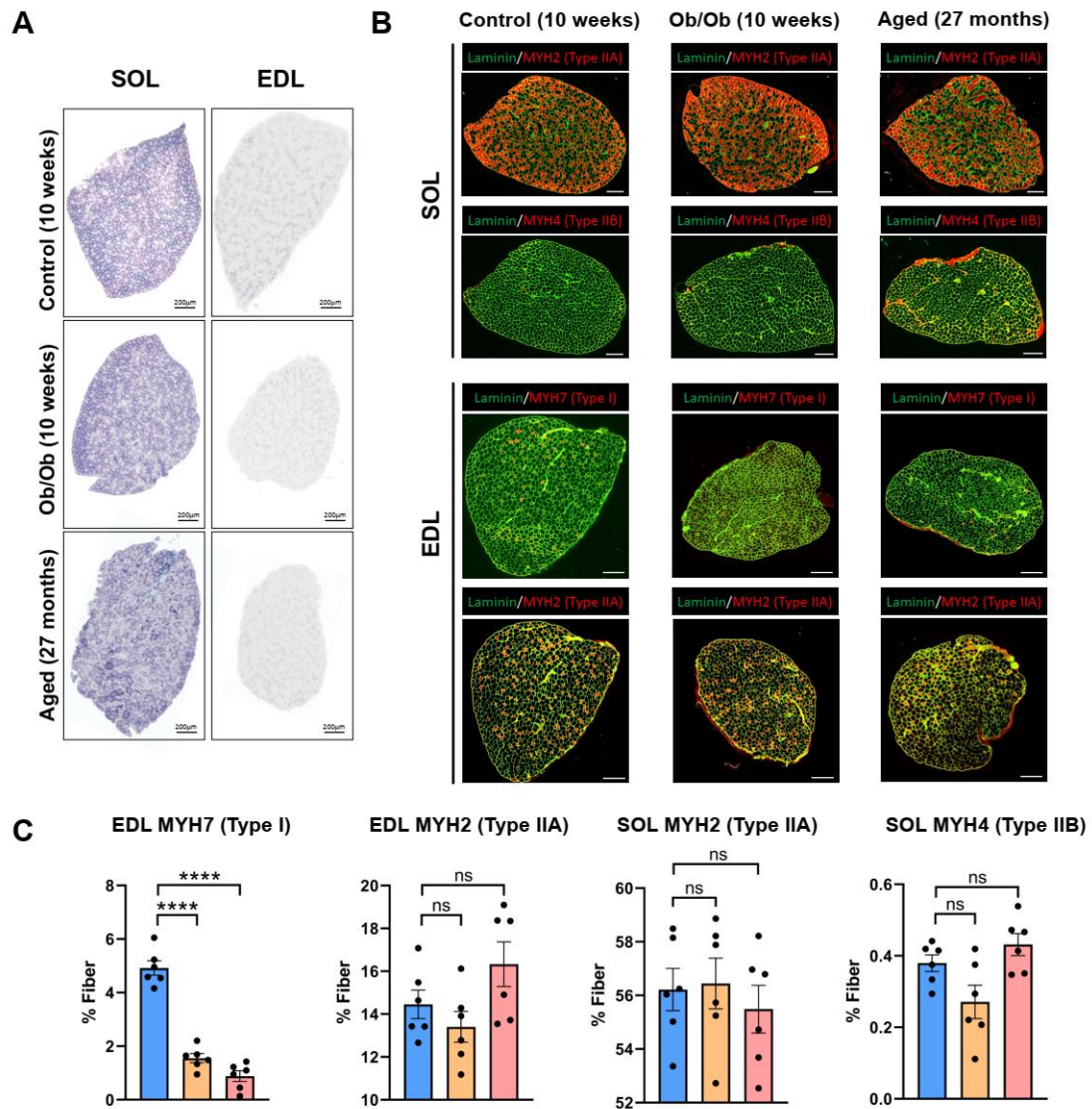




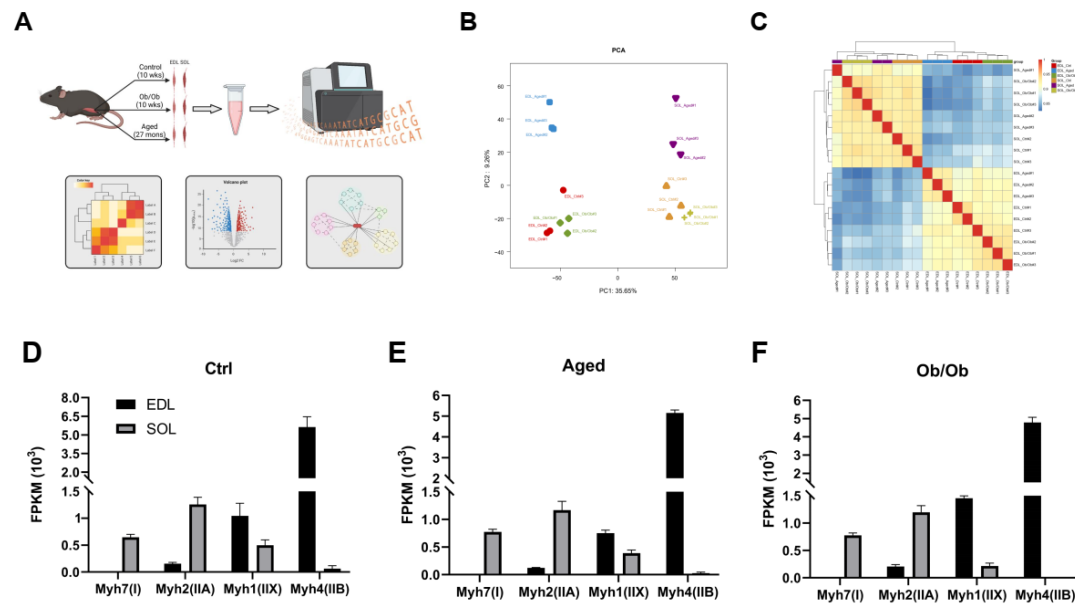
Supplementary Figure 1. Phenotype of control (10 weeks), Ob/Ob (10 weeks), and aged (27 months) C57BL/6 mice. **(A)** Body weight of control (10 weeks), Ob/Ob (10 weeks), and aged (27 months) mice. **(B)** Grip strength, data were normalized by body weight. **(C)** Glucose tolerance test (GTT). n=3-6; ** indicated $P < 0.01$, *** indicated $P < 0.001$, **** indicated $P < 0.0001$; # indicated control group vs. Ob/Ob group, $P < 0.05$.



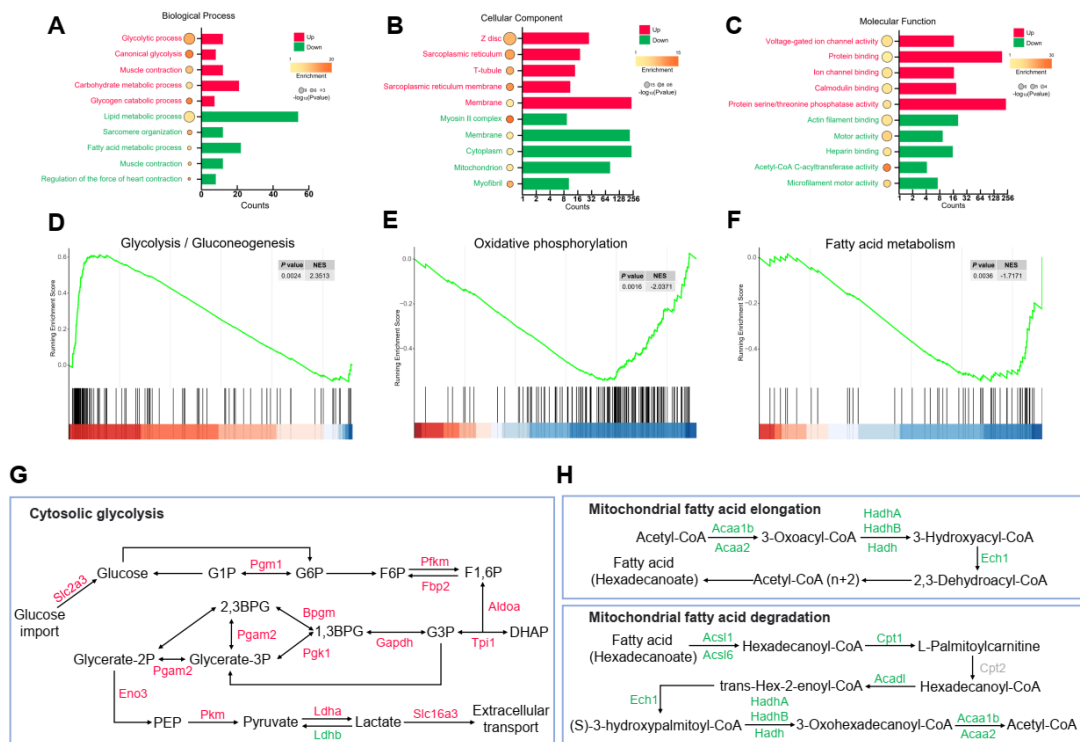
Supplementary Figure 2. Aging and obesity lead to a decrease in the proportion of oxidative fibers in quadriceps. **(A)** Immunofluorescence staining of type I and IIA fibers in control, aged, and Ob/Ob mice. **(B)** Statistical results of the proportion of type I fibers. **(C)** Statistical results of the proportion of type IIA fibers. $n=5-6$, ** indicated $P<0.01$, **** indicated $P<0.0001$.



Supplementary Figure 3. SOL predominantly consists of oxidative muscle fibers, while EDL is primarily composed of glycolytic fibers. **(A)** SDH staining of TA sections in control, aged, and Ob/Ob mice. **(B)** Immunofluorescence staining of type IIA and IIB for SOL, and type I and IIA for EDL. **(C)** Statistical results of the proportion of type I, IIA, and IIB fibers in EDL or SOL. $n=6$, **** indicated $P<0.0001$.

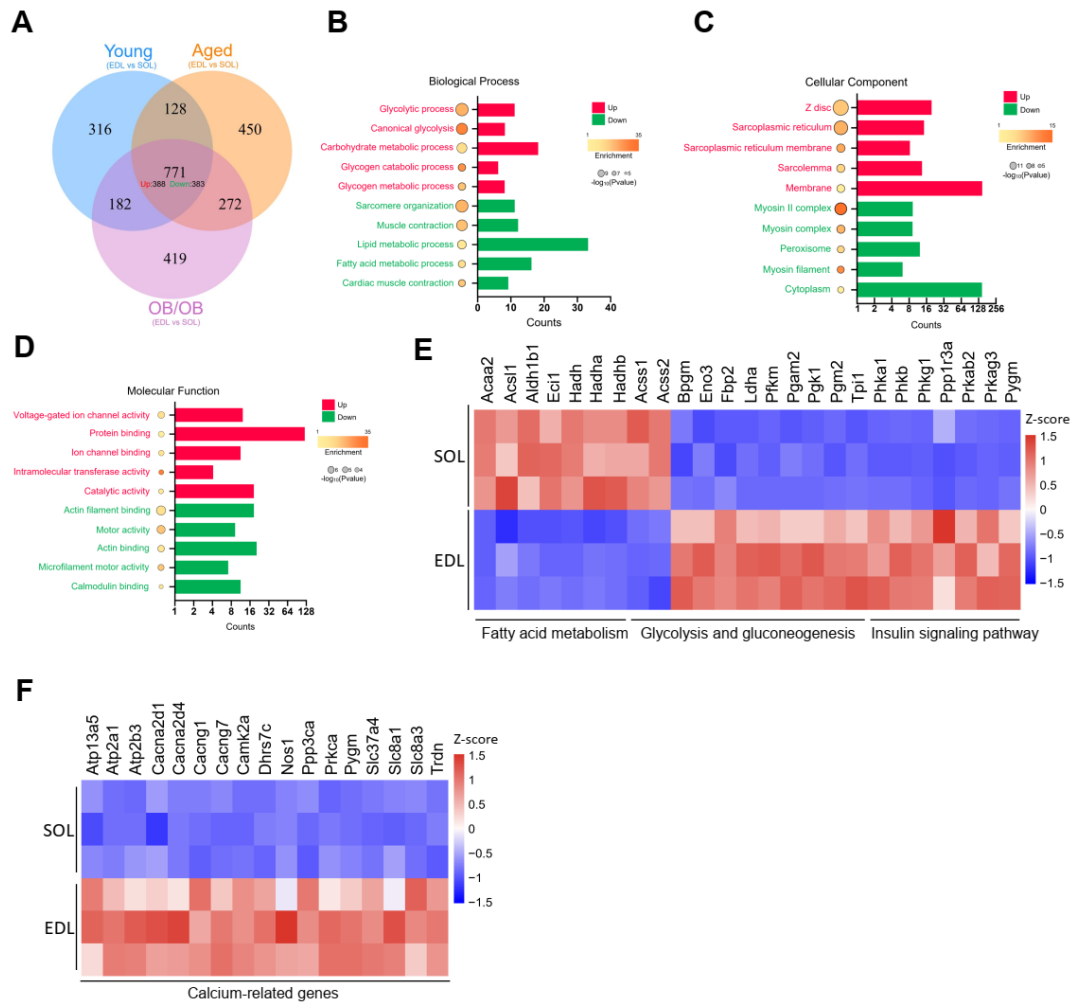


Supplementary Figure 4. Schematic diagram of RNA sequencing. **(A)** Scheme representing the mice model, experimental set-up and assays employed. Figure is created by Biorender. **(B-C)** Principal component analysis (PCA) and correlation heatmap illustrating the similarity between transcriptomic profiles generated from RNA-Seq samples. **(D-F)** Expression level of Myh1, Myh2, Myh4, and Myh7 in EDL and SOL from control, aged, and Ob/Ob mice analyzed by RNA-seq. n=3.

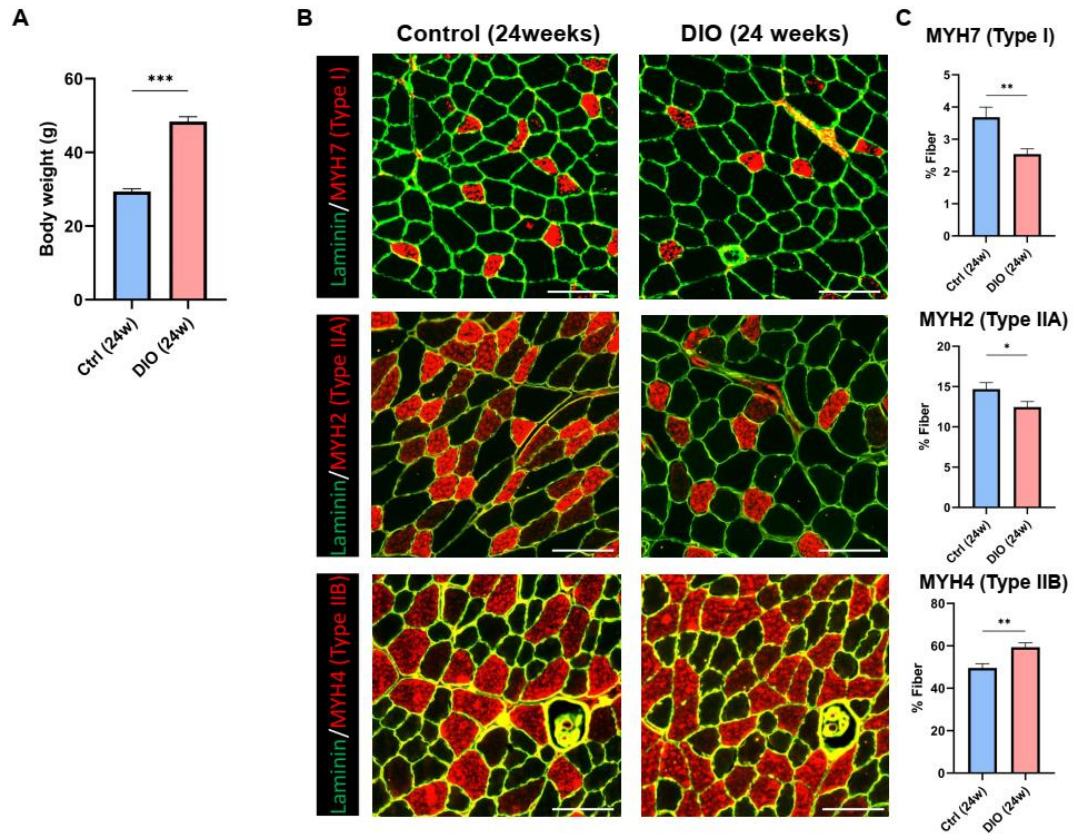


Supplementary Figure 5. RNA-Seq analysis of DEGs between EDL and SOL from control mice.

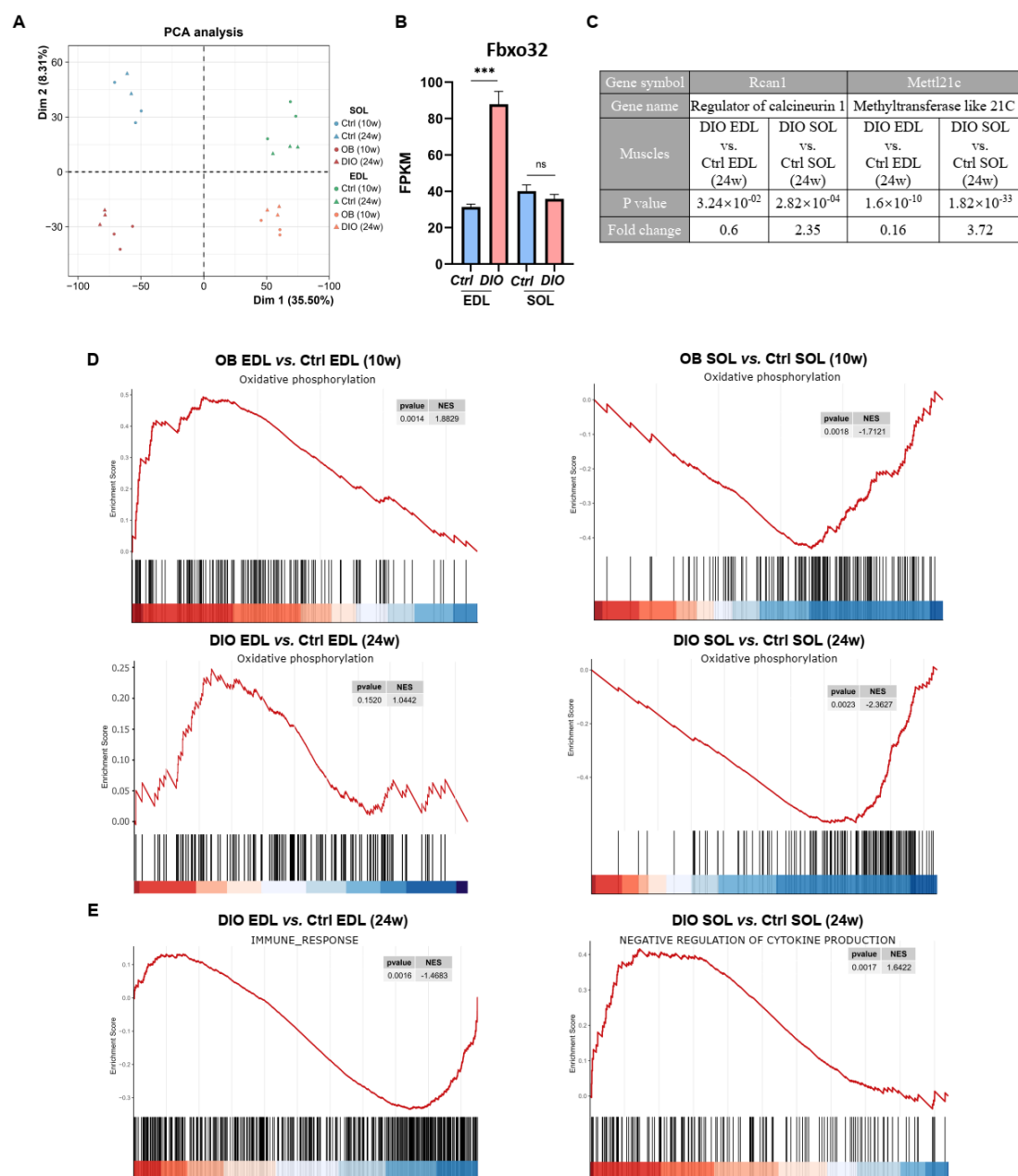
(A-C) GO analysis showing enriched biological processes, molecular function, and cellular component terms of DEGs between EDL and SOL from control mice. (D-F) Representative differences of transcriptome patterns between EDL and SOL generated by GSEA analysis. (G-H) DEGs involved in glycolysis and fatty acid metabolism among EDL and SOL.



Supplementary Figure 6. Identification of commonly expressed genes with muscle fiber type specificity across different groups. **(A)** The Venn plot demonstrated that there were 771 commonly expressed DEGs (677 protein-coding genes) between EDL and SOL that were shared among control, aged, and Ob/Ob mice. **(B-D)** GO analysis of these commonly expressed protein-coding gene DEGs. **(E-F)** Heatmap of genes involving in fatty acid metabolism, glycolysis and gluconeogenesis, insulin signaling pathway, and calcium homeostasis.

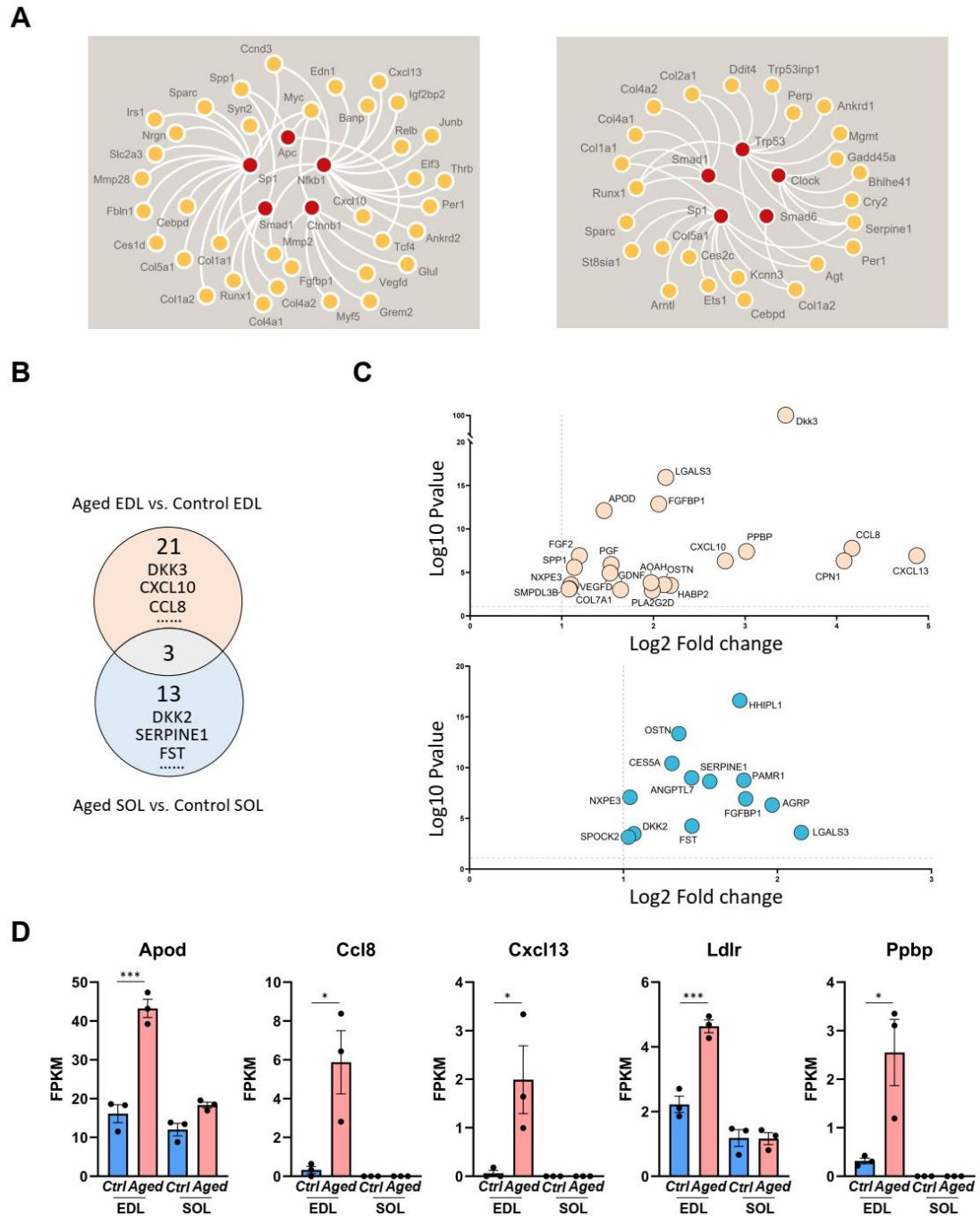


Supplementary Figure 7. Diet-induced obese (DIO) mice exhibited similar muscle fiber type changes in the TA as observed in Ob/Ob mice. **(A)** Body weight of DIO mice and littermate controls. **(B)** Immunofluorescence staining of MYH7, MYH2, MYH4 of TA sections. Scale bar: 100 μ m. **(C)** Statistical results of the proportion of type I, IIA, IIB fibers. Data are expressed as percentages; n=3 or 6; * indicated $P<0.05$, ** indicated $P<0.01$.

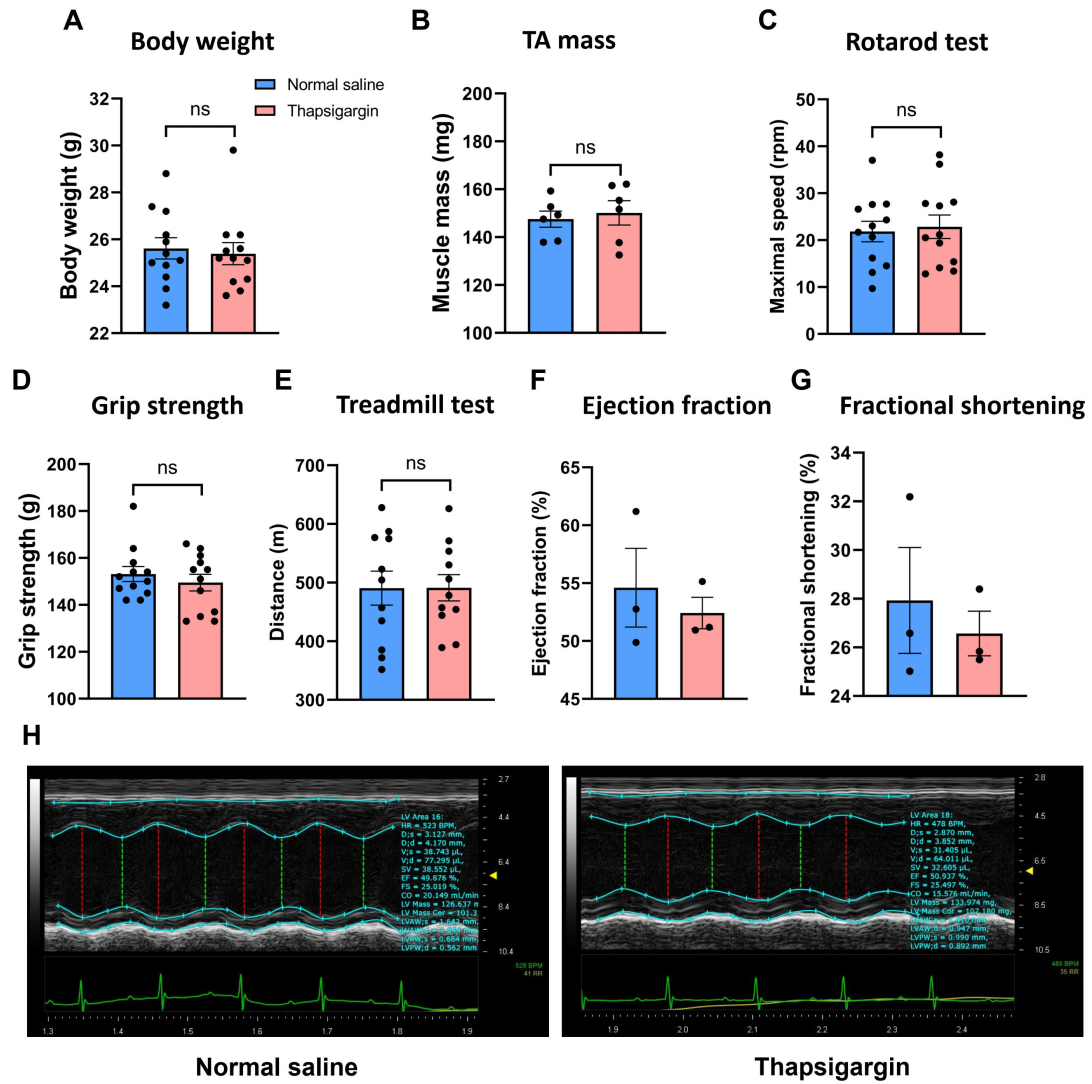


Supplementary Figure 8. DIO mice and Ob/Ob mice had similar overall transcriptome profiles.

(A) PCA analysis. (B) PFKM of Fbxo32 gene in EDL and SOL of DIO and control mice. (C) Expression level of Rcan1 and Mettl21c in EDL and SOL of DIO and control mice. (D) GSEA analysis of oxidative phosphorylation in DIO and Ob/Ob mice. (E) GSEA analysis of immune-related pathways.



Supplementary Figure 9. Aged EDL exhibited a pronounced inflammatory phenotype. **(A)** Transcriptional regulatory network analysis of DEGs between control and aged EDL, as well as between control and aged SOL. **(B)** Venn diagram illustrating the shared upregulated genes encoding secreted proteins. **(C)** Volcano plot displaying the upregulated genes encoding secreted proteins. **(D)** The top 5 upregulated genes encoding proteins related to inflammation.



Supplementary Figure 10. One month of thapsigargin injections had no detrimental effects on muscle and cardiac function. **(A)** Body weight. **(B)** TA mass. **(C)** Rotarod test. **(D)** Grip strength test. **(E)** Treadmill test. **(F)** Ejection fraction. **(G)** Fractional shortening. **(H)** Representative images of mouse cardiac ultrasound show no significant difference in cardiac function between the thapsigargin-injected and normal saline groups. n=3 or 12.

Supplementary Table 1. Top 10 upregulated and 10 downregulated DEGs with an average FPKM>10 in EDL or SOL.

Gene symbol	Gene name	Log ₂ FC (WT_EDL vs. WT_SOL)	Regulation	P value
Myh4	Myosin heavy chain 4	6.470873738	Up	4.85×10 ⁻¹⁸
Mss51	MSS51 mitochondrial translational activator	5.831504388	Up	4.23×10 ⁻³⁵
Mstn	Myostatin	5.534096604	Up	7.77×10 ⁻⁹⁶
Pvalb	Parvalbumin	5.475860327	Up	5.41×10 ⁻⁷⁰
Actn3	Actinin alpha 3	5.315657531	Up	1.17×10 ⁻²⁰
Mybpc2	myosin binding protein C, fast-type	5.06095736	Up	6.42×10 ⁻¹⁹
Tspan8	Tetraspanin 8	4.963672006	Up	1.97×10 ⁻⁶⁵
Mybph	Myosin binding protein H	4.507055986	Up	2.61×10 ⁻⁰⁹
Kcnc4	Potassium voltage gated channel, Shaw-related subfamily, member 4	4.48659624	Up	7.49×10 ⁻¹²⁶
Actc1	Actin, alpha, cardiac muscle 1	4.446559479	Up	4.30×10 ⁻⁴⁰
Myl3	Myosin light chain 3	-8.089464945	Down	1.43×10 ⁻²⁶
Myh7	Myosin heavy chain 7	-7.527033817	Down	3.34×10 ⁻¹⁰
Tnni1	Troponin I, skeletal, slow 1	-6.999636907	Down	6.32×10 ⁻¹⁷
Tnnc1	Troponin C, cardiac/slow skeletal	-6.943041949	Down	1.30×10 ⁻²⁴
Strit1	Small transmembrane regulator of ion transport 1	-6.748057324	Down	0.002
Tnnt1	Troponin T1, skeletal, slow	-6.707614629	Down	2.42×10 ⁻³⁵
Myl2	myosin, light polypeptide 2, regulatory, cardiac, slow	-6.560322344	Down	2.15×10 ⁻¹¹⁷
Atp2a2	ATPase, Ca ²⁺ transporting, cardiac muscle, slow twitch 2	-5.917923961	Down	2.74×10 ⁻¹⁸⁰
Sim2	Single-minded family bHLH transcription factor 2	-5.189073277	Down	2.38×10 ⁻⁴⁵
Tpm3	Tropomyosin 3	-5.026340947	Down	5.80×10 ⁻¹⁸²

Data was derived from control EDL vs. control SOL.