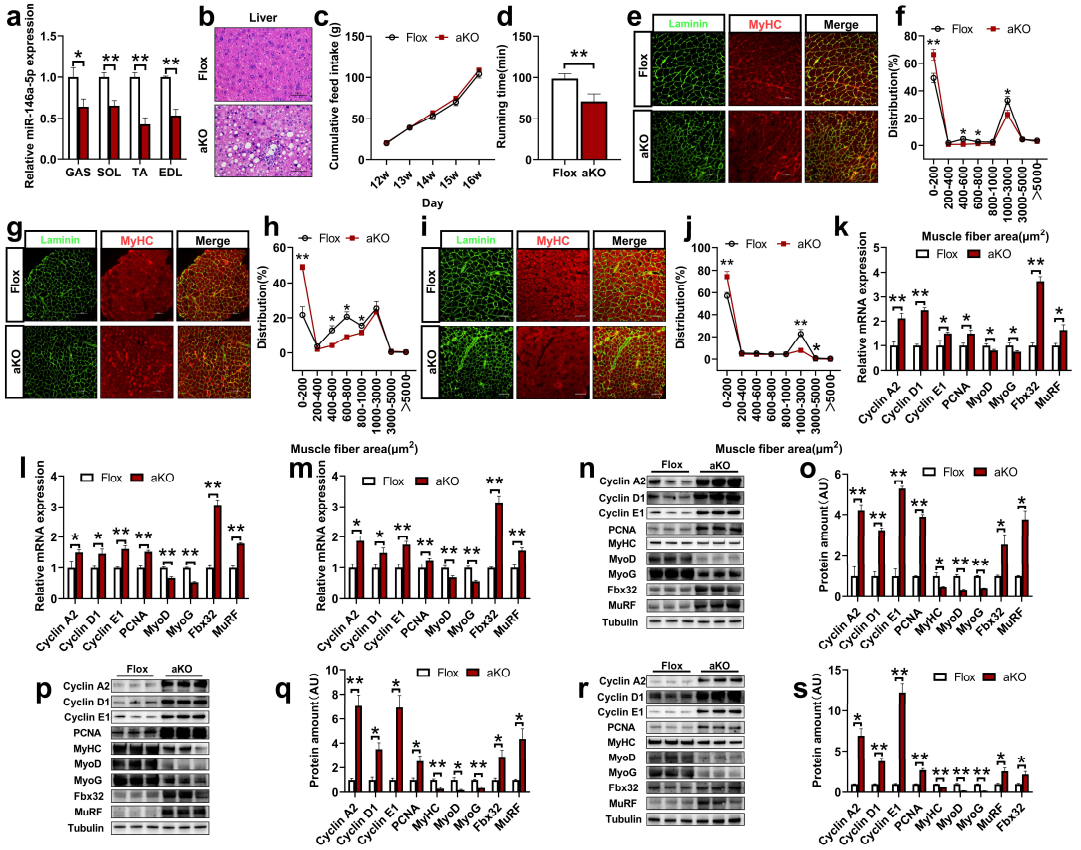
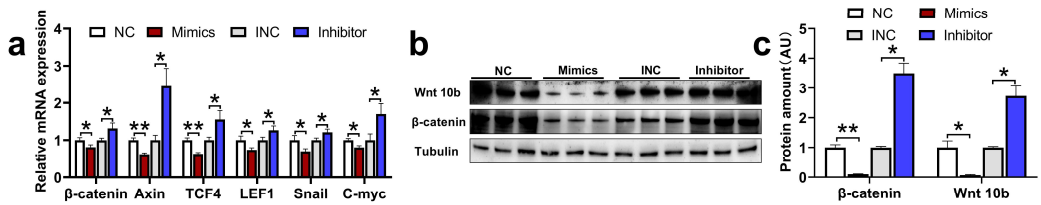


Supplementary Figure 1

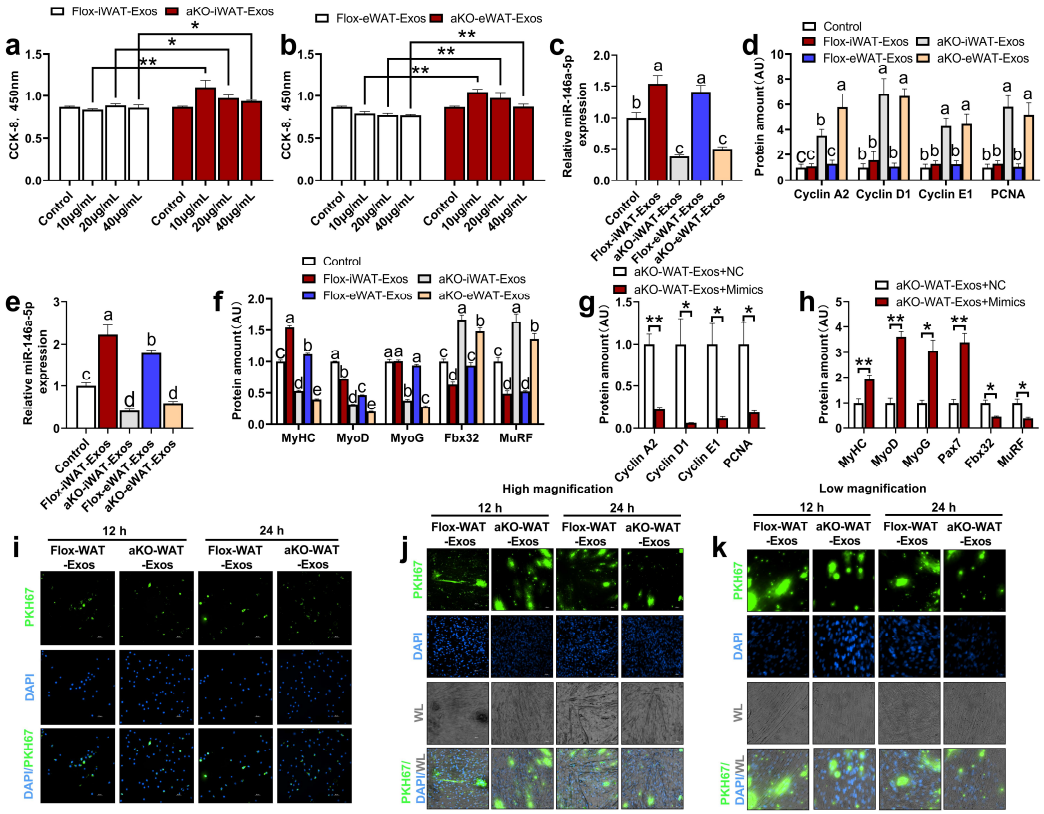
Effects of adipose-specific miR-146a-5p knockout on endurance exercise capacity, metabolism, and glucose homeostasis in mice. (a) The expression of miR-146a-5p gene in GAS, SOL, TA, EDL (n = 3). (b) Representative H&E staining of Liver from mice (scale bar = 50 μm). (c) Accumulate feed intake of mice (n = 8). (d) Running time at low speed (n = 6). (e) Representative cross sections GAS fiber immunofluorescent MyHC staining (scale bar = 100 μm). (f) Frequency histogram of fiber cross-sectional area (n = 6). (g) Representative cross-sections SOL fiber immunofluorescent MyHC staining (scale bar = 100 μm). (h) Frequency histogram of fiber cross-sectional area (n = 6). (i) Representative cross sections EDL fiber immunofluorescent MyHC staining (scale bar = 100 μm). (j) Frequency histogram of fiber cross-sectional area (n = j). (k) RT-qPCR analysis for Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyoD, MyoG, Fbx32, and MuFR in the GAS muscles of Flox

and aKO mice (n = 6). (l) RT-qPCR analysis for Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyoD, MyoG, Fbx32, and MuFR in the SOL muscles of Flox and aKO mice (n = 6). (m) RT-qPCR analysis for Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyoD, MyoG, Fbx32, and MuFR in the EDL muscles of Flox and aKO mice (n = 6). (n, o) The protein levels and statistical analyses results of Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyHC, MyoD, MyoG, Fbx32, and MuFR measured by Western blot in the GAS muscles of Flox and aKO mice (n = 3). (p, q) The protein levels and statistical analyses results of Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyHC, MyoD, MyoG, Fbx32, and MuFR measured by Western blot in the SOL muscles of Flox and aKO mice (n = 3). (r, s) The protein levels and statistical analyses results of Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyHC, MyoD, MyoG, Fbx32, and MuFR measured by Western blot in the EDL muscles of Flox and aKO mice (n = 3). Values are presented as means ± SEM, $*P < 0.05$, and $**P < 0.01$, according to the non-paired Student's t-test or one-way ANOVA between individual groups.



Supplementary Figure 2

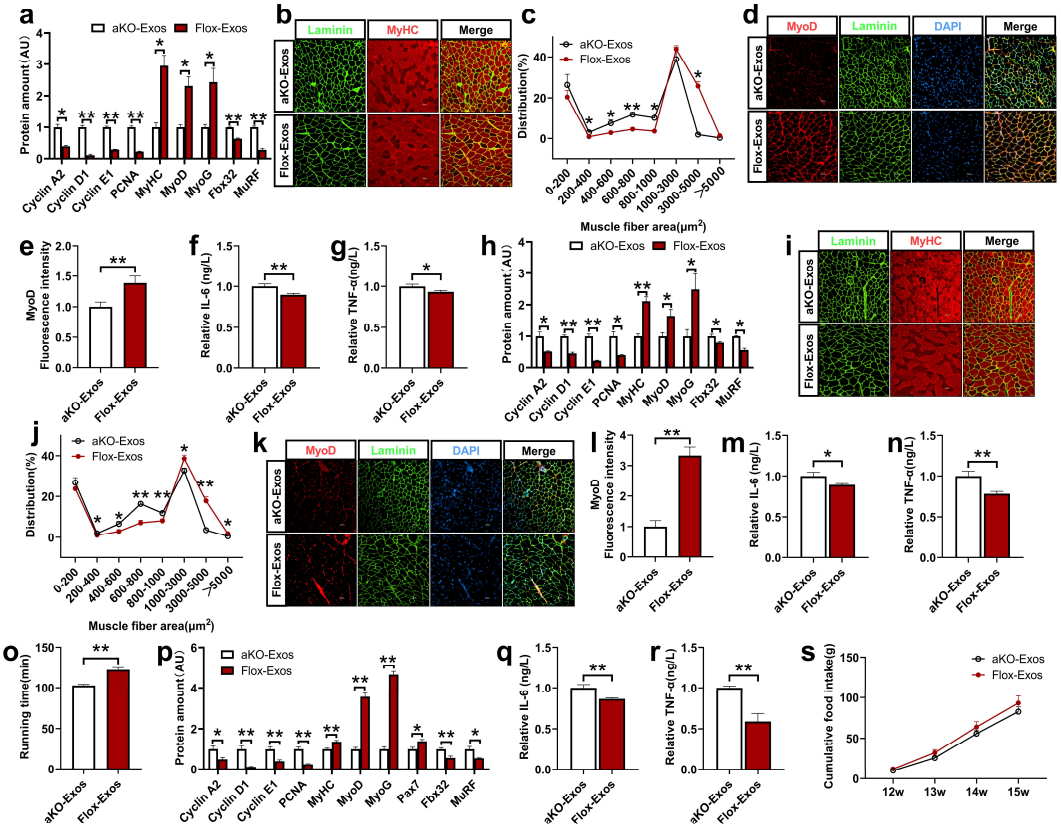
miR-146a-5p inhibits proliferation and promotes differentiation in C2C12 cells. (a) RT-qPCR analysis for β-catenin, Axin, TCF4, LEF1, Snail, and C-myc in C2C12 cells proliferation of transfected with miR-146a-5p mimics/inhibitor (n = 6). (b, c) The protein levels and the statistical analyses results of Wnt10b and β-catenin by Western blot in C2C12 cells proliferation transfected with miR-146a-5p mimics/inhibitor (n = 3). Values are presented as means ± SEM, $*P < 0.05$, and $**P < 0.01$, according to the non-paired Student's t-test or one-way ANOVA between individual groups.



Supplementary Figure 3

A miR-146a-5p mimic reversed the myotube atrophy of C2C12 cells induced by aKO-WAT-Exos. (a) CCK-8 result of C2C12 treated with Control (PBS), Flox-iWAT-Exos (10 μg/mL, 20 μg/mL, 30 μg/mL), aKO-iWAT-Exos (10 μg/mL, 20 μg/mL, 30 μg/mL). (b) CCK-8 result of C2C12 treated with Control (PBS), Flox-eWAT-Exos (10 μg/mL, 20 μg/mL, 30 μg/mL), aKO-eWAT-Exos (10 μg/mL, 20 μg/mL, 30 μg/mL). (c) The expression of miR-146a-5p gene in proliferation-C2C12 treated with Flox-iWAT-Exos, aKO-iWAT-Exos, Flox-eWAT-Exos, aKO-eWAT-Exos (n = 6). (d) The statistical analyses results of Cyclin A2, Cyclin D1, Cyclin E1, PCNA by Western blot in C2C12 of treated with Contral (PBS), Flox-iWAT-Exos, aKO-iWAT-Exos, Flox-eWAT-Exos, aKO-eWAT-Exos (n = 3). (e) The expression of miR-146a-5p gene in differentiation-C2C12 treated with Flox-iWAT-Exos, aKO-iWAT-Exos, Flox-eWAT-Exos, aKO-eWAT-Exos (n = 6). (f) The statistical analyses results of MyHC, MyoD, MyoG, Fbx32, and MuFR by Western blot in differentiation-

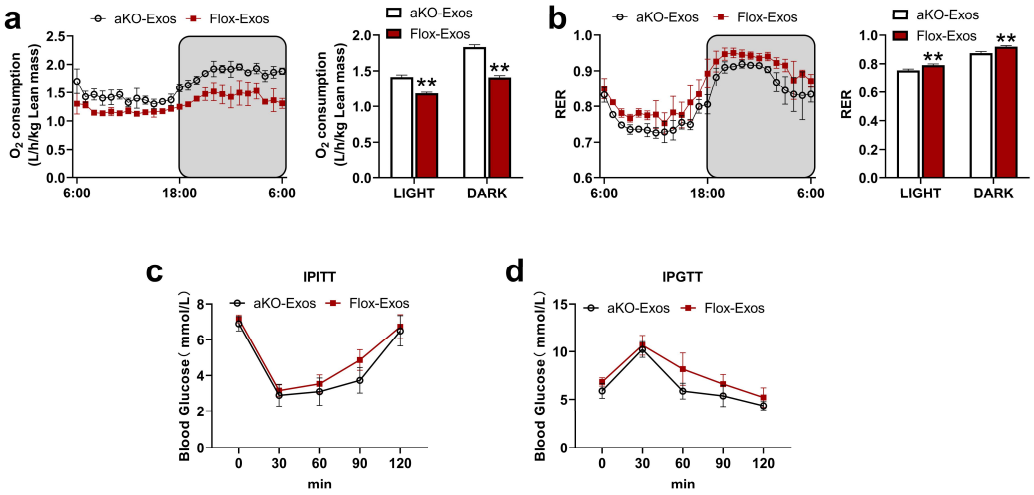
C2C12 of treated with Control (PBS), Flox-iWAT-Exos, aKO-iWAT-Exos, Flox-eWAT-Exos, aKO-eWAT-Exos. (n = 3). (g) The protein statistical results of Cyclin A2, Cyclin D1, Cyclin E1, and PCNA in proliferation-C2C12 treated with aKO-WAT-Exos+NC and aKO-WAT-Exos+Mimics (n = 3). (h) The protein statistical results of MyHC, MyoD, MyoG, Pax7, Fbx32, and MuFR by Western blot in differentiation-C2C12 cells treated with aKO-WAT-Exos+NC and aKO-WAT-Exos+Mimics (n = 3). (i) Fluorescence microscopy showed the uptake of PKH67-labelled exosomes by proliferation-C2C12 cells in 12/24 hours (scale bar = 50 μm). (j, k) Fluorescence microscopy high/low magnification showed the uptake of PKH67-labelled exosomes by differentiation-C2C12 cells in 12/24 hours (scale bar = 50 μm). Values are presented as means ± SEM, $*P < 0.05$, and $**P < 0.01$, according to the non-paired Student's t-test or one-way ANOVA between individual groups.



Supplementary Figure 4

WAT-derived exosomes miR-146a-5p may be involved in muscle atrophy. (a) The protein statistical results of Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyHC, MyoD, MyoG, Fbx32, and MuFR measured by Western blot in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 12 h (n = 3). (b, c) Representative cross sections TA fiber immunofluorescent MyHC staining in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 12 h (scale bar = 50 μm) (n = 6). (d, e) Representative cross sections TA fiber immunofluorescent MyoD staining in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 12 h (scale bar = 50 μm) (n = 6). (f, g) ELISA analysis for IL-6 and TNF-α in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 12 h (n = 6). (h) The protein statistical results of Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyHC, MyoD, MyoG, Fbx32, and MuFR measured by Western blot in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 24 h (n = 3). (i, j) Representative cross

sections TA fiber immunofluorescent MyHC staining in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 24 h (scale bar = 50 μm) (n = 6). (k, l) Representative cross sections TA fiber immunofluorescent MyoD staining in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 24 h (scale bar = 50 μm) (n = 6). (m, n) ELISA analysis for IL-6 and TNF-α in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 24 h (n = 6). (o) Running time at low speed (n=3). (p) The protein statistical results of Cyclin A2, Cyclin D1, Cyclin E1, PCNA, MyHC, MyoD, MyoG, Fbx32, and MuFR measured by Western blot in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 24 d (n = 3). (q, r) ELISA analysis for IL-6 and TNF-α in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 24 d (n = 4). (s) Accumulate feed intake of mice (n = 4). Values are presented as means ± SEM, $*P < 0.05$, and $**P < 0.01$, according to the non-paired Student's t-test or one-way ANOVA between individual groups.



86

87 **Supplementary Figure 5**

88 **WAT-derived exosomes miR-146a-5p may be involved in muscle atrophy.** (a) The O_2

89 consumption (VO_2) (n = 4). (b) RER (n = 4). (c, d) IPITT and IPGTT blood glucose changes in aKO

90 mice (n = 6).

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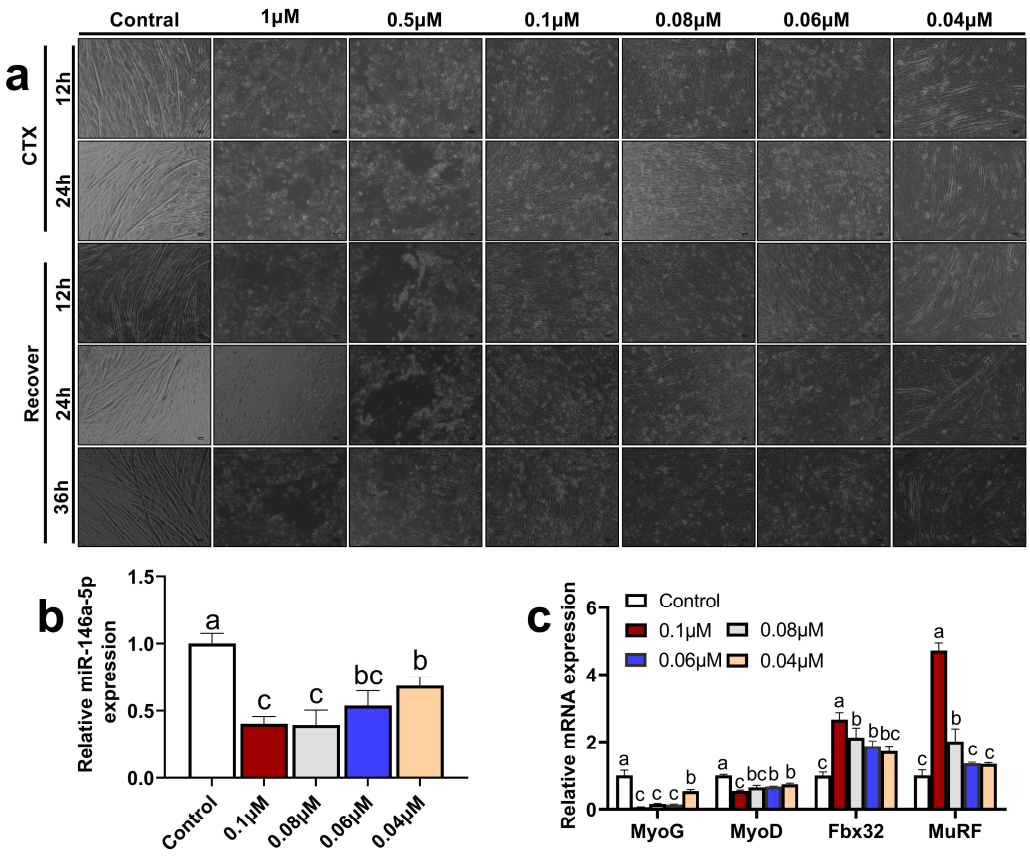

92

93 **Supplementary Figure 6**

94 **miR-146a-5p is sufficient to alleviate or treat muscle atrophy in vitro.** (a) Representative muscle

95 fiber of C2C12 cells following treatment with Control (PBS), CTX (1 μM, 0.5 μM, 0.1 μM, 0.08

96 μM, 0.06 μM, 0.04 μM) after 12 h/24 h and recover 12 h /24 h /36 h (scale bar = 50 μm). (b) The

97 expression of miR-146a-5p gene in C2C12 cells following treatment with Control (PBS), CTX (1

98 μM, 0.5 μM, 0.1 μM, 0.08 μM, 0.06 μM, 0.04 μM) (n = 4). (c) RT-qPCR analysis for MyoD, MyoG,

99 Fbx32, and MuFR in C2C12 cells following treatment with Control (PBS), CTX (1 μM, 0.5 μM,

100 0.1 μM, 0.08 μM, 0.06 μM, 0.04 μM) (n = 6). Values are presented as means ± SEM, *P < 0.05,

101 and **P < 0.01, according to the non-paired Student's t-test or one-way ANOVA between

102 individual groups.

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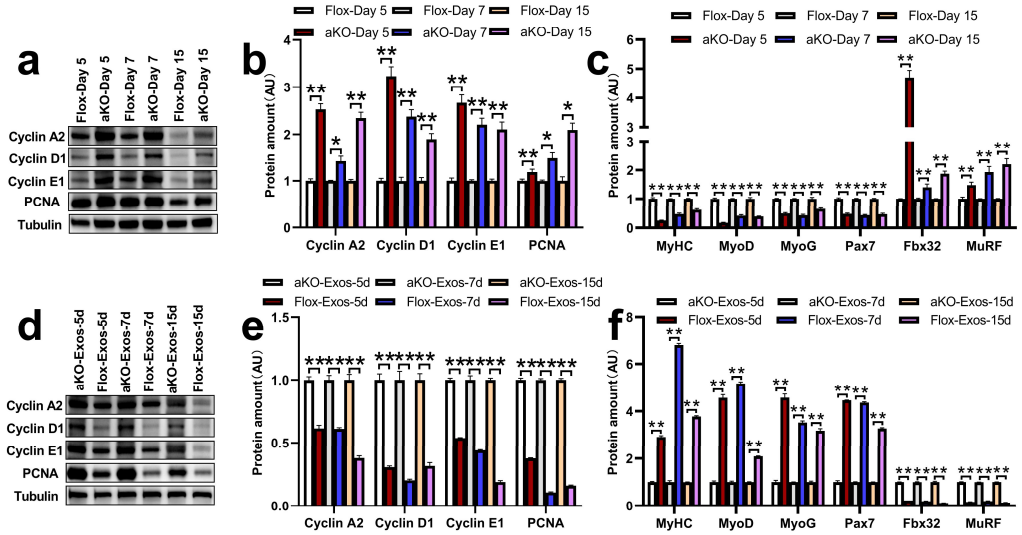
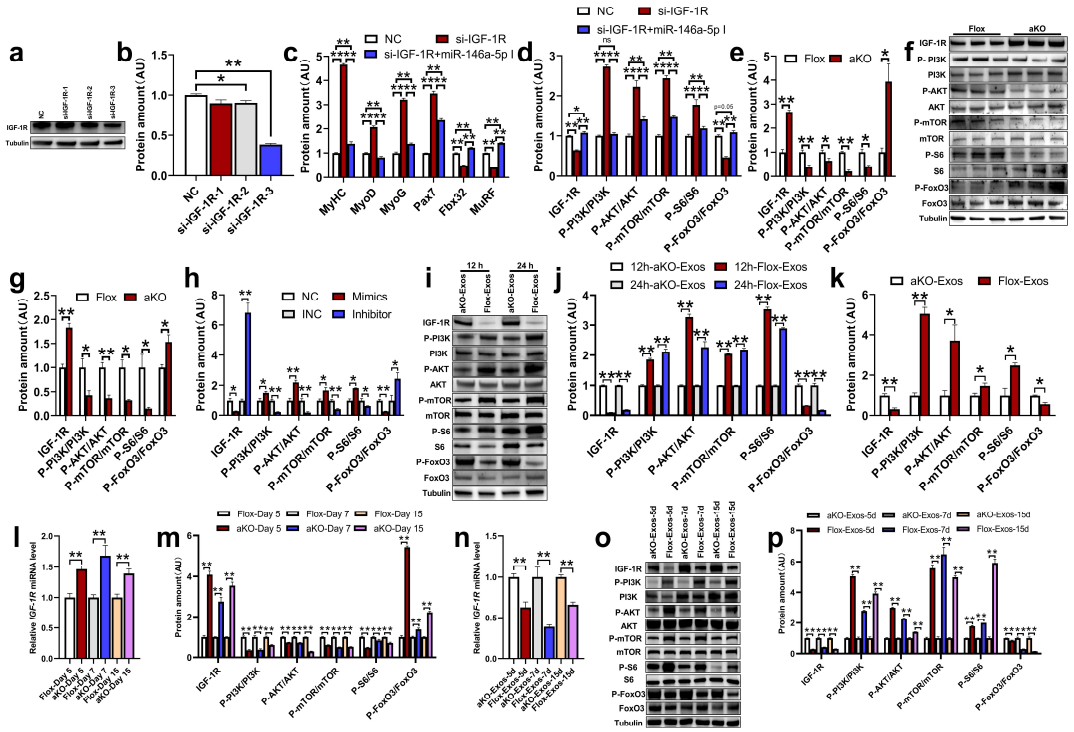


104

105 **Supplementary Figure 7**

106 **Adipose-derived miR-146a-5p is indispensable in muscle atrophy and repair.** (a, b) The protein
107 levels and statistical results of Cyclin A2, Cyclin D1, Cyclin E1, and PCNA were measured by
108 Western blot in TA muscle cross-sections from injured (5, 7, and 15 days after CTX injection) Flox
109 and aKO mice (n = 3). (c) The protein statistical results of MyHC, MyoD, MyoG, Pax7, Fbx32, and
110 MuFR were measured by Western blot in TA muscle cross-sections from injured (5, 7, and 15 days
111 after CTX injection) Flox and aKO mice (n = 3). (d, e) The protein levels and statistical results of
112 Cyclin A2, Cyclin D1, Cyclin E1, and PCNA were measured by Western blot in TA muscle cross-
113 sections from injured (5, 7, and 15 days after CTX, aKO-Exos, and Flox-Exos injection) aKO mice
114 (n = 3). (f) The protein statistical results of MyHC, MyoD, MyoG, Pax7, Fbx32, and MuFR
115 measured by Western blot in TA muscle cross-sections from injured (5, 7, and 15 days after CTX,
116 aKO-Exos, and Flox-Exos injection) aKO mice (n = 3). Values are presented as means ± SEM, *P
117 < 0.05, and **P < 0.01, according to the non-paired Student's t-test or one-way ANOVA between
118 individual groups.

119



Supplementary Figure 8

miR-146a-5p prevents muscle atrophy by targeting IGF-1R. (a, b) C2C12 cells were transfected with IGF-1R siRNA for 48 h, and IGF-1R was detected by Western Blot (n = 3). (c) The statistical results of MyHC, MyoD, MyoG, Pax7, Fbx32, and MuFR by Western blot in C2C12 cells following transfection with siRNA-IGF-1R, co-treatment with siRNA-IGF-1R and miR-146a-5p inhibitor (n = 3). (d) The statistical results of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3, and FoxO3 measured by Western blot in C2C12 cells following transfection with siRNA-IGF-1R, co-treatment with siRNA-IGF-1R and miR-146a-5p inhibitor (n = 3). (e) The statistical results of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3, and FoxO3 measured by Western blot in the TA muscles of Flox and aKO mice (n = 3). (f, g) The protein levels and statistical results of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3 and FoxO3 measured by Western blot in the GAS muscles of Flox and aKO mice (n = 3). (h) The statistical results of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3

and FoxO3 by Western blot in differentiation-C2C12 cells transfected with miR-146a-5p mimics/inhibitor (n = 3). (i, j) The protein levels and statistical results of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3 and FoxO3 measured by Western blot in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 12 h/24 h (n = 3). (k) The statistical results of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3, and FoxO3 measured by Western blot in the aKO mice TA muscles injections of aKO-Exos and Flox-Exos for 24 d (n = 3). (l) RT-qPCR analysis for IGF-1R in TA muscle cross-sections from injured (5, 7, and 15 days after CTX injection) Flox and aKO mice (n = 4). (m) The protein levels of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3, and FoxO3 measured by Western blot in TA muscle cross-sections from injured (5, 7 and 15 days after CTX injection) Flox and aKO mice (n = 3). (n) RT-qPCR analysis for IGF-1R in TA muscle cross-sections from injured (5, 7, and 15 days after CTX, aKO-Exos, and Flox-Exos injection) aKO mice (n = 4). (o-p) The protein levels of IGF-1R, P-PI3K, PI3K, P-AKT, AKT, P-mTOR, mTOR, P-S6, S6, P-FoxO3, and FoxO3 measured by Western blot in TA muscle cross-sections from injured (5, 7 and 15 days after CTX, aKO-Exos, and Flox-Exos injection) aKO mice (n = 3). Values are presented as means ± SEM, $*P < 0.05$, and $**P < 0.01$, according to the non-paired Student's t-test or one-way ANOVA between individual groups.

Table S1. Primer sequences for genotypic identification

Gene	Forward (5’-3’)	Reverse (5’-3’)
Loxp-5	CTGCTCTTGCTGACGTGAAGAA	TTCCTAGAGTGACCCAGTTCTACATG
Loxp-6	GATGATCCCTCACTAACACTCTTTCTA	GAGTCACAGCAGCAAGAACCACTC
Adipose-Cre	GGATGTGCCATGTGAGTCTG	ACGGACAGAAGCATTTTCCA

Table S2. siRNA sequences for C2C12 cells transfection

Gene	Forward (5’-3’)	Reverse (5’-3’)
IGF-1R siRNA-1	GAAUCGAGUUUCUCAACGATT	UCGUUGAGAAACUCGAUUCTT
IGF-1R siRNA-2	GCUUCACAGUUUACUACAATT	UUGUAGUAAACUGUGAAGCTT
IGF-1R siRNA-3	CGGUAGCUGACACCUACAATT	UUGUAGGUGUCAGCUACCGTT
mmu-miR-146a-5p mimics	UGAGAACUGAAUUCCAUGGGUU	CCCAUGGAAUUCAGUUCUCAUU
mmu-miR-146a-5p inhibitor	AACCCAUGGAAUUCAGUUCUCA	

Table S3. Primer sequences for quantitative real-time PCR

Gene	Forward (5’-3’)	Reverse (5’-3’)
mmu-GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
mmu-U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
mmu-miR-146a-5p	GGGTGAGAACTGAATTCCA	CAGTGCGTGTCGTGGAGT

mmu-Cyclin A2	TTGTAGGCACGGCTGCTATGCT	GGTGCTCCATTCTCAGAACCTG
mmu-Cyclin B1	AGAGGTGGAACTTGCTGAGCCT	GCACATCCAGATGTTTCCATCGG
mmu-Cyclin D1	GCAGAAGGAGATTGTGCCATCC	AGGAAGCGGTCCAGGTAGTTCA
mmu-Cyclin E1	AAGCCCTCTGACCATTGTGTCC	CTAAGCAGCCAACATCCAGGAC
mmu-PCNA	CAAGTGGAGAGCTTGGCAATGG	GCAAACGTTAGGTGAACAGGCTC
mmu-P21	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
mmu-β-catenin	GTTCGCCTTCATTATGGACTGCC	ATAGCACCCTGTTCCCGCAAAG
mmu-Axin	ATGGAGTCCCTCCTTACCGCAT	GTTCCACAGGCGTCATCTCCTT
mmu-TCF4	CCTGCGGATATAGACAGCACTTC	TGTCCAGGTACACCAGATCCCA
mmu-LEF1	ACTGTCAGGCGACACTTCCATG	GTGCTCCTGTTTGACCTGAGGT
mmu-Snail	TGTCTGCACGACCTGTGGAAAG	CTTCACATCCGAGTGGGTTTGG
mmu-C-myc	CCTGGTGCTCCATGAGGAGAC	CAGACTCTGACCTTTTGCCAGG
mmu-TNF-α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
mmu-IL-1β	GCTGCTTCCAAACCTTTGAC	AGCTTCTCCACAGCCACAAT
mmu-IL-6	AGTTGCCTTCTTGGGACTGA	CCTCCGACTTGTGAAGTGGT
mmu-MyoD	GCACTACAGTGGCGACTCAGAT	TAGTAGGCGGTGTCGTAGCCAT
mmu-MyoG	CCATCCAGTACATTGAGCGCCT	CTGTGGGAGTTGCATTCACTGG
mmu-Pax7	GTTCGGGAAGAAAGAGGACGAC	GGTTCTGATTCCACATCTGAGCC
mmu-Fbx32	CTTCTCGACTGCCATCCTGGAT	TCTTTTGGGCGATGCCACTCAG
mmu-MuRF	TACCAAGCCTGTGGTCATCCTG	ACGGAAACGACCTCCAGACATG
mmu-MyHC Ⅰ	GCTGGAAGATGAGTGCTCAGAG	TCCAAACCAGCCATCTCCTCTG
mmu-MyHC Ⅱa	GCGACTTGAAGTTAGCCCAGGA	CTCGTCCTCAATCTTGCTCTGC

mmu-MyHC Ⅱb	AGAGCCAAGAGGAAACTGGAGG	CTCGTCCTCAATCTTGCTCTGC
mmu-MyHC Ⅱx	AGAGCCAAGAGGAAACTGGAGG	CTCGTCCTCAATCTTGCTCTGC
mmu-IGF-1R	CGGGATCTCATCAGCTTCACAG	TCCTTGTTCGGAGGCAGGTCTA

Table S4. Primer sequences for reverse transcription

Gene	Sequences (5'-3')
mmu-miR-146a-5p RT	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAACCCA