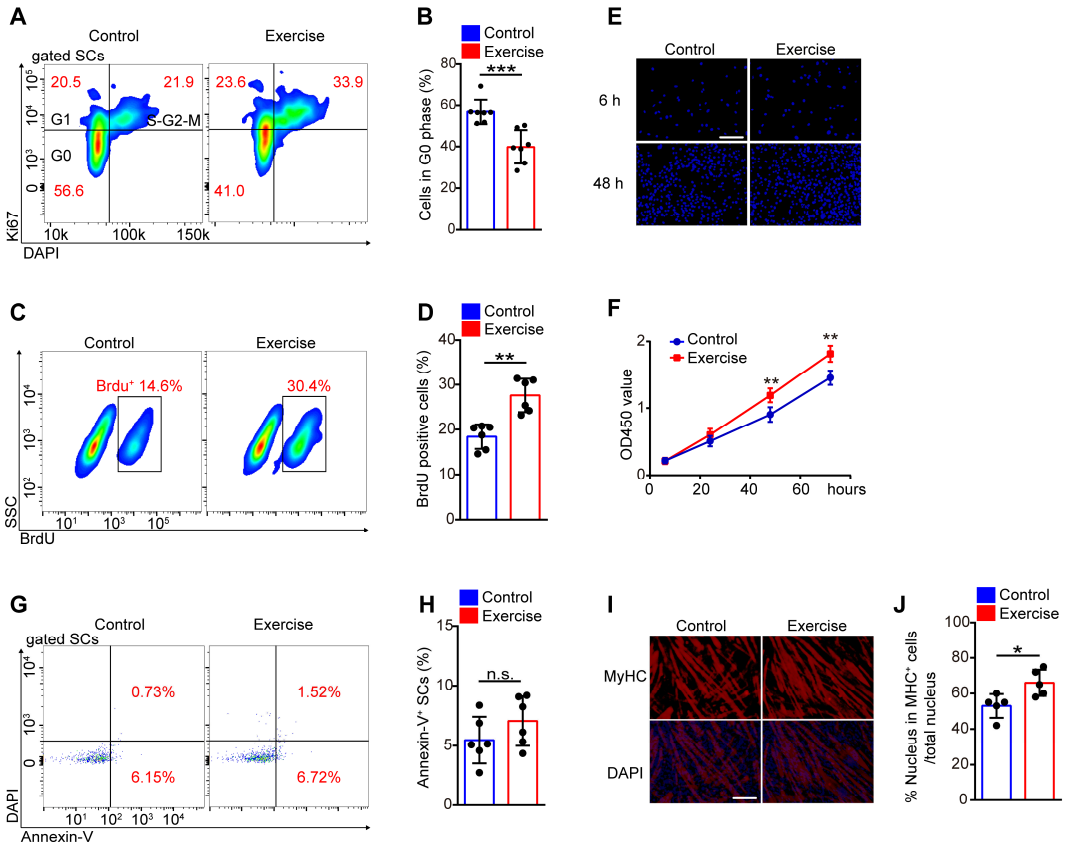
Supplementary Figures and Figure legends

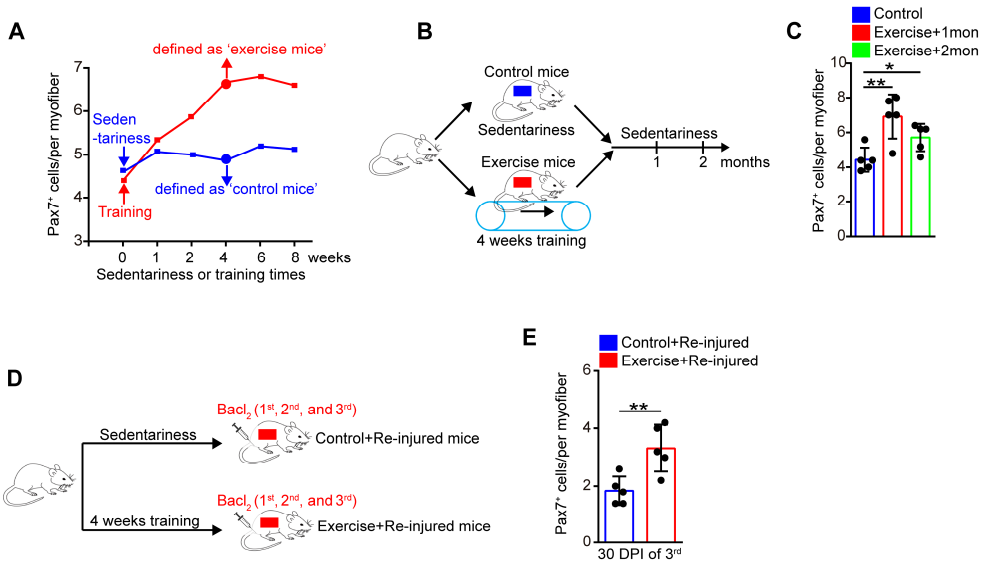
Figure S1



Supplementary Figure 1. The effect of exercise on proliferation, apoptosis, and differentiation of SCs

(**A**) The mononuclear cells isolated from skeletal muscles were stained with cell surface markers, DAPI and Ki67. The quiescent percentage of SCs was analyzed by flow cytometry. (**B**) Quantification of the percentage of G0 phase in SCs (n=7 mice). (**C**) SCs were isolated from mice and cultured *in vitro*. SCs were labeled with BrdU. Flow cytometry analysis was conducted to detected $BrdU^+$ cells. (**D**) The frequency of $BrdU^+$ SCs (n=6). **(E)** Representative fluorescent images of immunostaining for SCs cultured in *vitro*. SCs were stained with DAPI. Scale bars, 100 μm. (**F**) *In vitro* proliferation assay (CCK8) of cultured SCs (n=5). (**G**) The mononuclear cells isolated from skeletal muscles in mice were stained with SC markers, as well as Annexin-V and DAPI. The population of apoptotic SCs was analyzed by flow cytometry. (**H**) The percentage of apoptotic SCs (Annexin-V^+ SCs) (n=6 mice). (**I**) SCs were isolated from mice and cultured in differentiation medium for 72 hours. Immunofluorescence of MHC in SCs. Nucleus was stained with DAPI. Scale bar, 100μm. (**J**) Quantification the percentage of nucleus in MHC^+ cells/total cells (n=5). Error bars represent the means ± SD. *p<0.05, **p<0.01, ***p<0.001; n.s. no significance; Student's t-test.

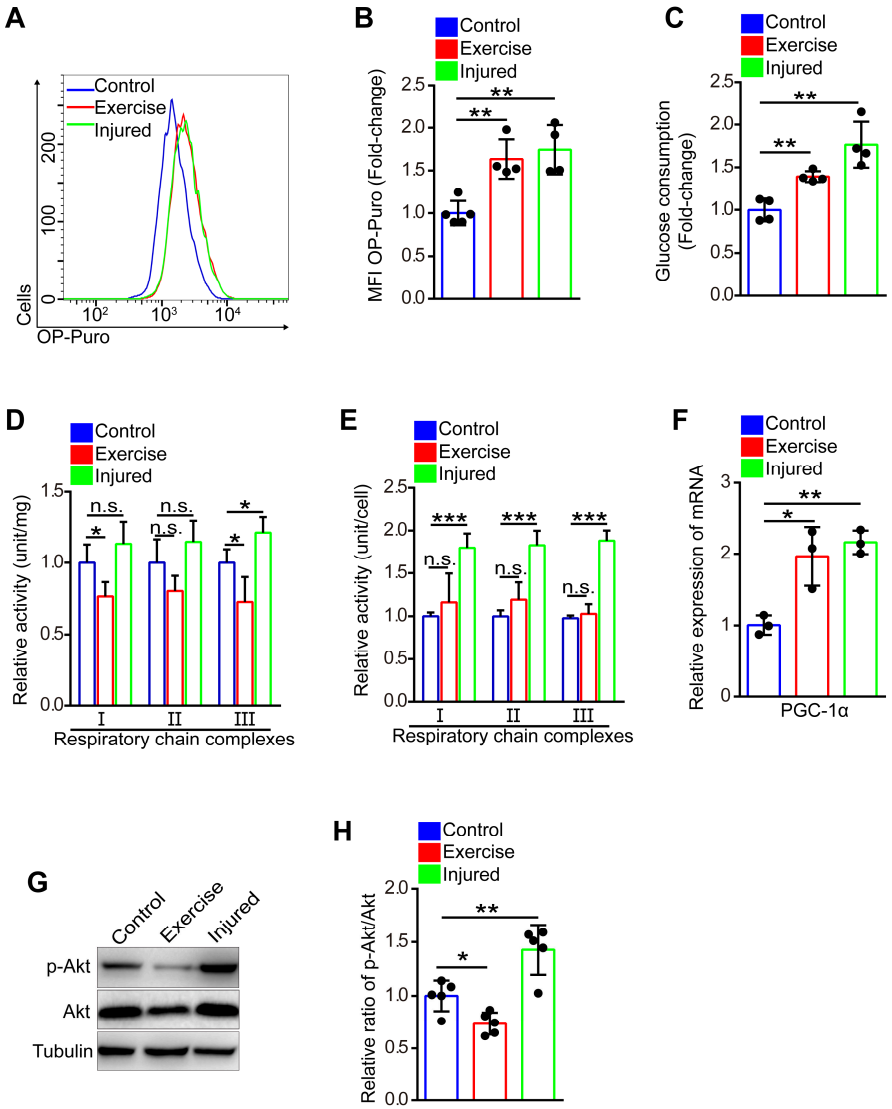
Figure S2



Supplementary Figure 2. The effect of exercise on SC maintenance

(A) The mice were subjected to sedentariness or training. Skeletal muscles were harvested depending on sedentariness or training time (0, 1, 2, 4, 6 and 8 weeks) and digested being single myofiber. Myofibers were fixed and stained by the Pax7 antibody, and the numbers of $Pax7^+$ SCs per myofiber were counted (n=3 mice, 20 myofibers per mouse). (**B**) A schematic illustration showing the design for the experiment. Sedentary mice defined as 'control mice'. Mice were trained for 4 weeks and then subjected to sedentariness for 1 month (defined as 'exercise+1mon') or 2 months (defined as 'exercise+2mon'). (**C**) Quantification of $Pax7^+$ SCs number per myofiber in mice (n=5 mice). (**D**) A schematic illustration showing the design for repeated muscle injuries (three times, interval for 14 days, repeated injured was defined as "Re-injured") (E) Quantification of $Pax7^+$ SCs number per myofiber of mice at 30 days post-injury (3^{rd}) (n=5 mice, 20 myofibers per mouse). Error bars represent the means ± SD. *p<0.05, **p<0.01; One-way ANOVA.

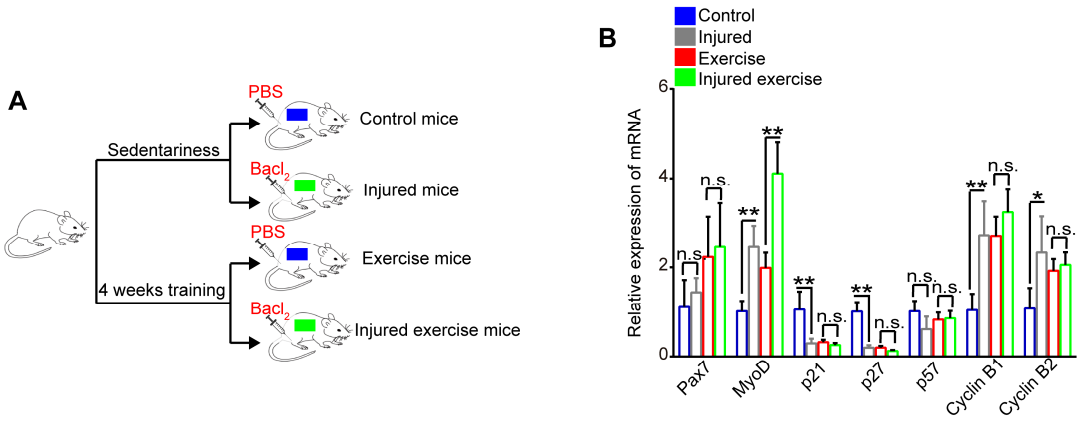
Figure S3



Supplementary Figure 3. The effect of exercise on SC metabolism and phosphorylation of Akt

(**A**) SCs were isolated from mice and cultured *in vitro*, the OP-Puro was added to the culture medium for 1 hour. Flow cytometry analysis was conducted to detect the intensity of OP-Puro. (**B**) The MFI analysis of OP-Puro in SCs (n=4). (**C**) SCs were isolated from mice and cultured for 24 hours *in vitro*, the change of glucose concentration in the culture medium was measured (n=4). (**D**, **E**) SCs were isolated from mice and cultured *in vitro*, the activity of respiratory chain complex was quantified as unit per mg protein (**D**), or unit per cell (**E**) (n=4). (**F**) SCs were sorted from mice. Total RNA of SCs was extracted and the expression of PGC-1α was assayed by qPCR (n=3 mice). (**G**) SCs were isolated from mice and cultured *in vitro*. The protein levels of p-Akt, Akt and Tubulin (loading control) were analyzed by western blot (n=3). Error bars represent the means ± SD. *p<0.05, **p<0.01, ***p<0.001; n.s. no significance; One-way ANOVA.

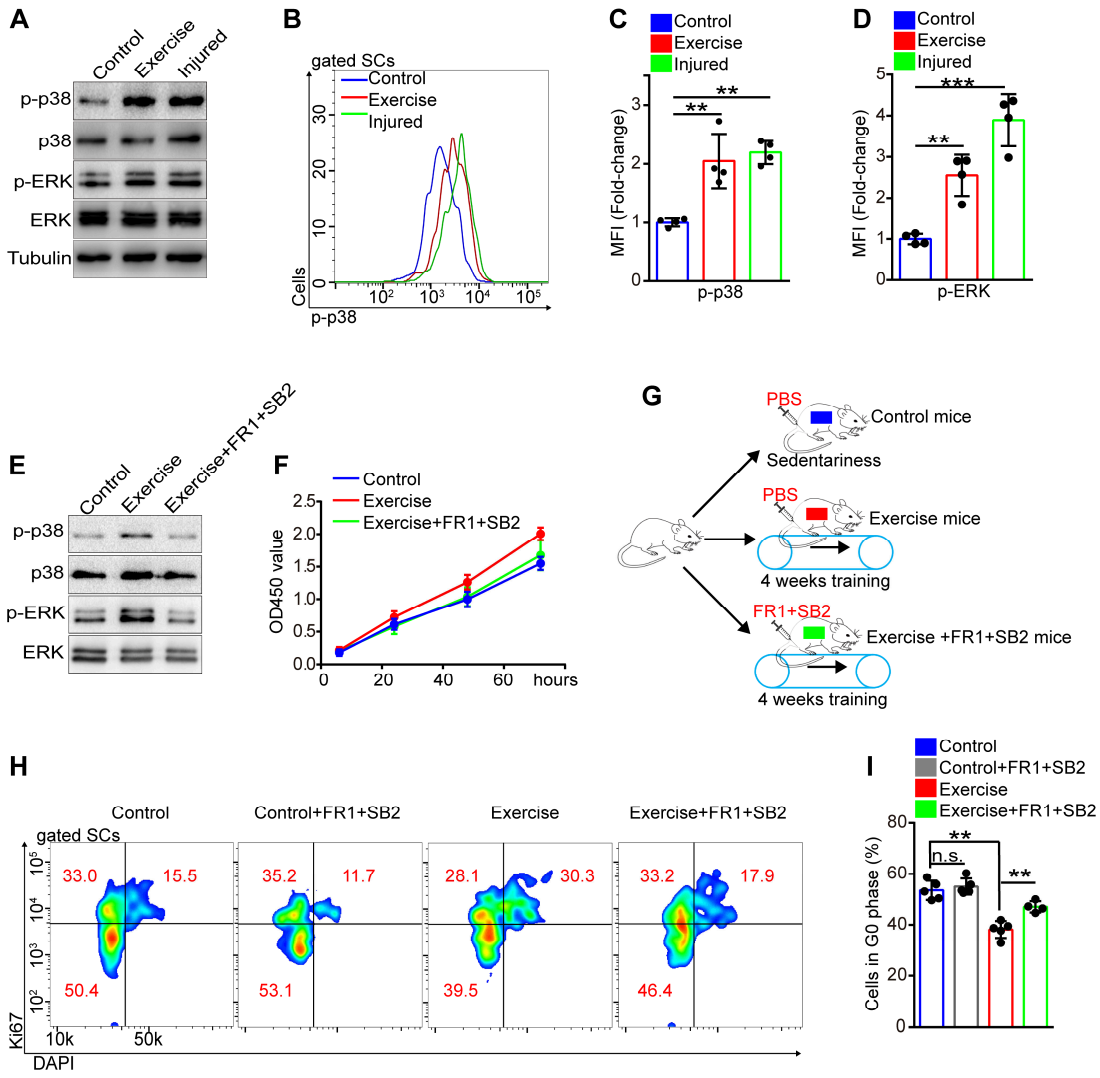
Figure S4



Supplementary Figure 4. The effect of exercise on gene expression of SCs

(**A**) A schematic illustration showing the design for muscle injury. Mice were subjected to sedentariness or training for 4 weeks, then PBS or $Bacl_2$ were injected into mice. (**B**) SCs were sorted from mice at 48 hours post-injection. Total RNA of SCs was extracted and the expression of indicated genes was assayed by qPCR (n=4 mice). Error bars represent the means ± SD. *p<0.05, **p<0.01; n.s. no significance; One-way ANOVA.

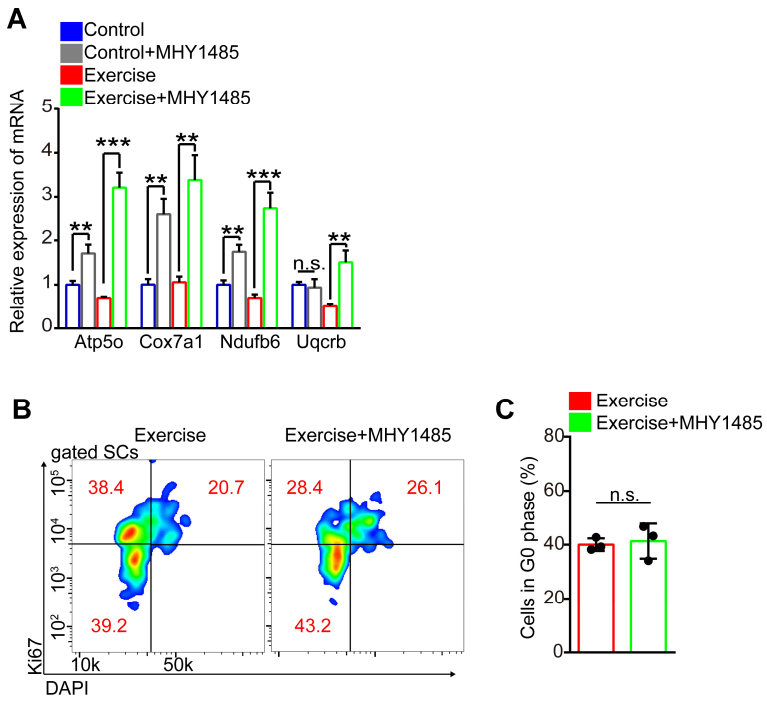
Figure S5



Supplementary Figure 5. Exercise promotes SCs cell cycling by MAPK pathway
(A) SCs were isolated from mice and cultured *in vitro*. The protein levels of p-p38, p38, p-ERK, ERK and Tubulin (loading control) were analyzed by western blot (n=3). (B) The mononuclear cells isolated from skeletal muscles were stained with SC markers and p-p38 fluorescent antibody. The intensity of p-p38 was analyzed by flow cytometry. (**C**) The MFI analysis of p-p38 in SCs in mice (n=4 mice). (**D**) The MFI analysis of p-ERK in SCs in mice (n=4 mice). (**E**) SCs isolated from control and exercise mice were cultured *in vitro* with the treatment of inhibitors of p38 (SB203580, SB2) and ERK (FR180204, FR1) for 24 hours. The protein levels of p-p38, p38, p-ERK, and ERK were analyzed by western blot (n=3). (**F**) *In vitro* proliferation assay (CCK8) of cultured SCs (n=5). (**G**) A schematic illustration showing the design for the treatment of FR1 and SB2. Sedentary mice were defined as "control mice" that injected with PBS. For experimental preciseness, "exercise mice" received PBS injection, and mice were defined as "exercise+FR1+SB2 mice" that subjected to 4 weeks of training and synchronized FR1 and SB2 injection. (**H**) The mononuclear cells isolated from skeletal muscles were stained with SC markers,

DAPI and Ki67. (**I**) Quantification of the percentage of G0 phase in SCs (n=4-5 mice). Error bars represent the means ± SD. *p<0.05, **p<0.01, ***p<0.001; n.s. no significance; One-way ANOVA.

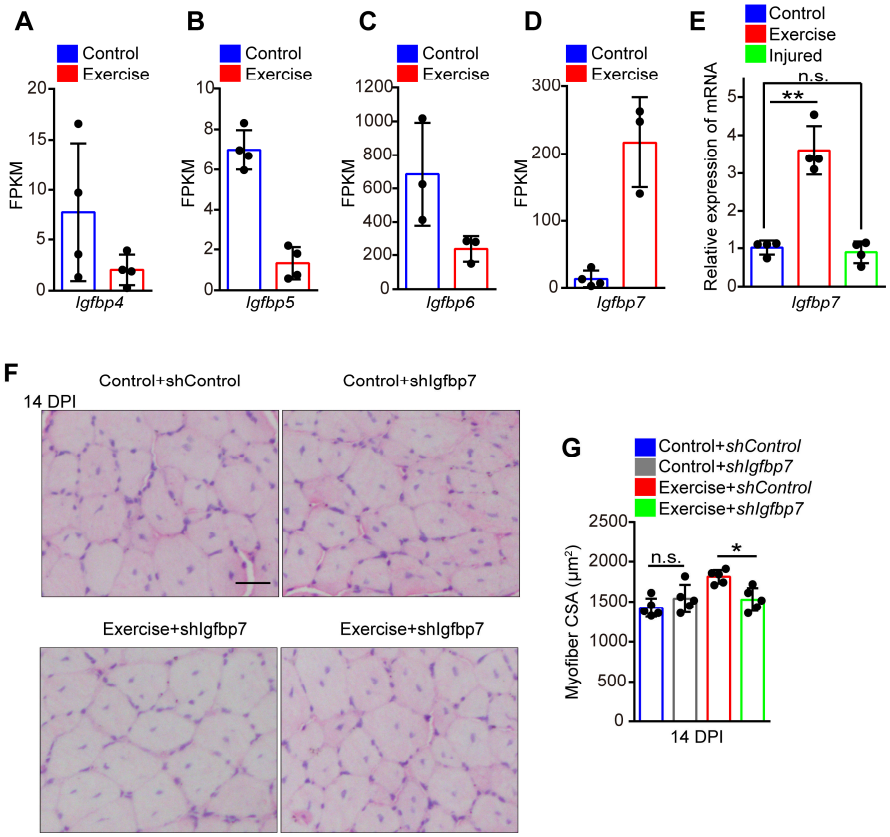
Figure S6



Supplementary Figure 6. The effect of MHY1485 on gene expression and cell cycle of SCs

(**A**) SCs were sorted from mice with or without MHY1485 treatment. Total RNA of SCs was extracted and the expression of indicated genes was assayed by qPCR (n=4 mice). (**B**) The mononuclear cells isolated from skeletal muscles were stained with SC markers, DAPI and Ki67. (**C**) Quantification of the percentage of G0 phase in SCs (n=3 mice). Error bars represent the means ± SD. **p<0.01, ***p<0.001; n.s. no significance; One-way ANOVA.

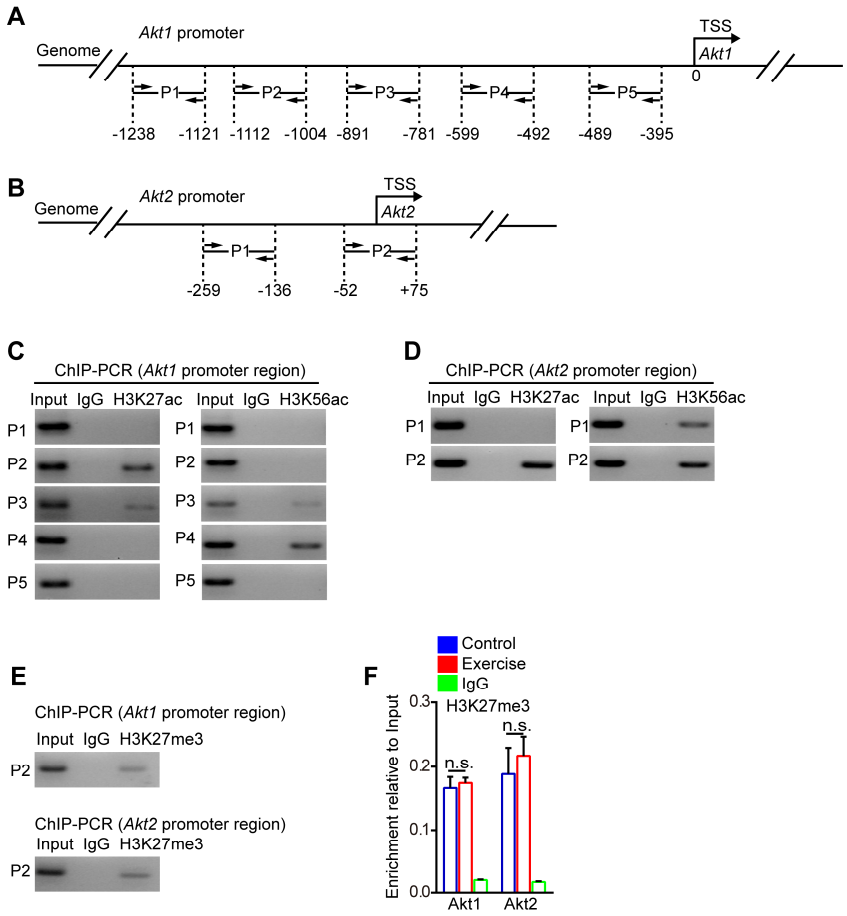
Figure S7



Supplementary Figure 7. The effect of knock-down of Igfbp7 in SCs on muscle regeneration

(**A-D**) The reads per kilobase per million (FPKM) value of Igfbp4, Igfbp5, Igfbp6, and Igfbp7 of SCs (n=3-4 mice, data from RNA-Sequence). (**E**) SCs were sorted from mice. Total RNA of SCs was extracted and the expression of Igfbp7 was assayed by qPCR (n=4 mice). (**F**) HE staining of the cross-sections of TA of control and exercise mice at 14 DPI. Scale bar, 100μm. (**G**) Average CSA of TA of control and exercise mice at 14 DPI (n=5 mice). Error bars represent the means ± SD. *p<0.05, **p<0.01; n.s. no significance; One-way ANOVA.

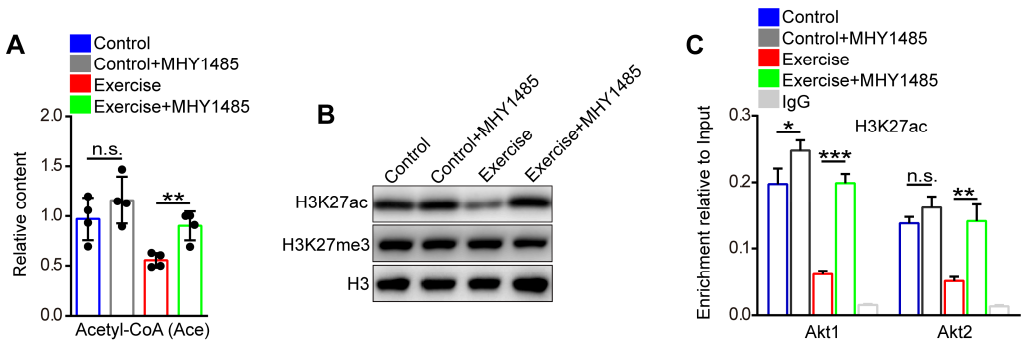
Figure S8



Supplementary Figure 8. ChIP-PCR analysis of H3K27ac and H3K56ac at promoters regions of Akt

(**A**, **B**) Schematic diagram of the upstream promoter regions of the Akt1 and Akt2 genes. Potential binding regions of H3K27ac and H3K56ac are shown: P1 (−1238 ~ −1121), P2 (−1112 ~ −1004), P3 (−891 ~ −781), P4 (−599~−492), and P5 (−489~−395) in Akt1 promoter region (**A**); and P1 (−259 ~ −136) and P2 (−52 ~ +75) in Akt2 promoter region (**B**). (**C**, **D**) Endogenous binding of H3K27ac and H3K56ac to the promoter regions of Akt1 (**C**) and Akt2 (**D**). IgG was used as a negative control. (**E**) Endogenous binding of H3K27me3 to the promoter regions of Akt1 and Akt2. (**F**) SCs were isolated and cultured *in vitro*. Quantitative ChIP-PCR was utilized to detect the binding of H3K27me3 at promoter regions of Akt1/2 in SCs. IgG served as a negative control. Enrichment relative to the input was shown (n = 4). Error bars represent the means ± SD. n.s. no significance; One-way ANOVA.

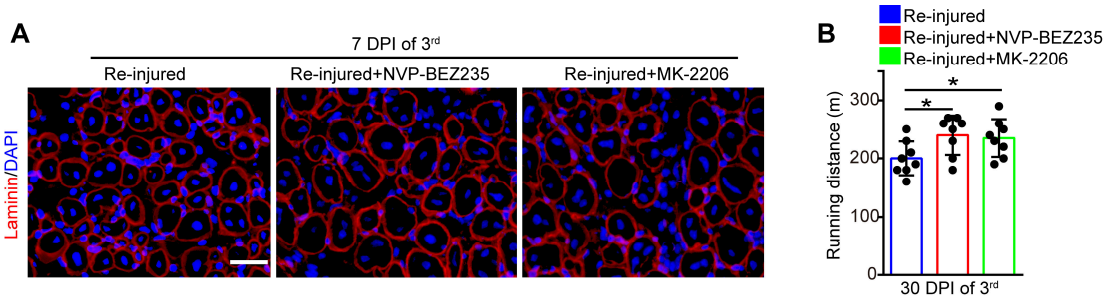
Figure S9



Supplementary Figure 9. The effect of MHY1485 treatment on acetylation of H3K27

(A) SCs were isolated from mice with or without MHY1485 treatment and cultured *in vitro*. The intracellular acetyl-CoA (Ace) levels of SCs were analyzed (n=4). (**B**) SCs were isolated from mice and cultured *in vitro*. The protein levels of H3K27ac, H3K27me3 and H3 (loading control) were analyzed by western blot (n=3). (**C**) SCs were isolated and cultured *in vitro*. Quantitative ChIP-PCR was utilized to detect the binding of H3K27ac at promoter regions of Akt1/2 in SCs. IgG served as a negative control. Enrichment relative to the input was shown (n = 4). Error bars represent the means ± SD. **p*<0.05, ***p*<0.01, ****p*<0.001; n.s. no significance; One-way ANOVA.

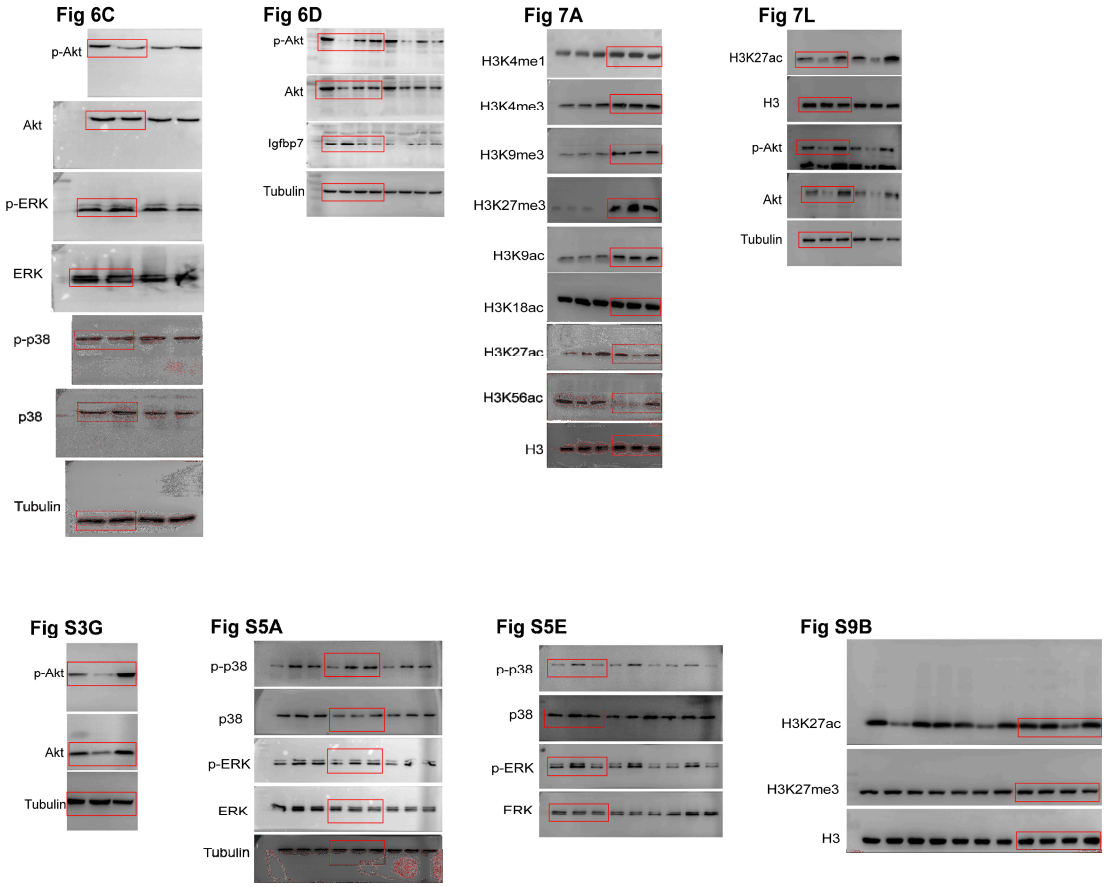
Figure S10



Supplementary Figure 10. The effect of NVP-BEZ235 and MK-2206 on muscle regeneration

(A) Immunofluorescence of Laminin and DAPI on cross-sections of TA of mice at 7 DPI of 3rd injury. Scale bars, 100 μm. (**B**) Mice were acclimated to running on the treadmill until exhaustion. The maximum running distances of mice at 30 DPI of 3rd injury were measured (n=8 mice). Error bars represent the means ± SD. *p<0.05; One-way ANOVA.

Figure S11



Supplementary Figure 11. Uncropped figures of western blot

Supplementary Table

Table S1: The sequences of primers

Primers for RT-PCR	
Pax7-F	GCTACCAGTACAGCCAGTATG
Pax7-R	GTAGGCTTGTCCCGTTTCC
MyoD-F	CGCTCCAACTGCTCTGATG
MyoD-R	ACACAGCCGCACTCTTC
P16-F	CTCTGGCTTTCGTGAACATG
P16-R	TCGAATCTGCACCGTAGTTG
P21-F	CTTGCACTCTGGTGTCTGAG
P21-R	GCACTTCAGGGTTTTCTCTTG
P27-F	TGGACCAAATGCCTGACTC
P27-R	GGGAACCGTCTGAAACATTTTC
P57-F	CAGGACGAGAATCAAGAGCAG
P57-R	CGACGCCTTGTTCTCCTG
Cyclin B1-F	CTGACCCAAACCTCTGTAGTG
Cyclin B1-R	CCTGTATTAGCCAGTCAATGAGG
Cyclin B2-F	CCTCAGAACACCAAAGTACCAG
Cyclin B2-R	CCTTCATGGAGACATCCTCAG
Acly-F	AAGCCTACATTGCAGACCTG
Acly-R	TTGACACCTCCAAGATCACAG
Hagh-F	TGGGCTGAAGGTTTATGGAG
Hagh-R	CAGATGTGTCCCGAAGTATGG
Grhpr-F	AGGTGGAACAGTGGAATTCG
Grhpr-R	GATGACTCTGAGGTTGGCTC
Ldha-F	ATGAAGGACTTGGCGGATG
Ldha-R	TGGAGTTCGCAGTTACACAG
Ldhd-F	ACGCTTTTCCTGGAGTTCC
Ldhd-R	CGCTGCCCAAAGTTCATTG
Acpy2-F	GGAGAAGCTAAGAAACGAGGTC
Acpy2-R	GTCGATGCGAGAACTAGGAC
Acaca-F	AAGGCTATGTGAAGGATGTGG
Acaca-R	CTGTCTGAAGAGGTTAGGGAAG
Idh3a-F	ACAAGACCCATCCCAGTTTG
Idh3a-R	GATTCAAAGATGGCAACACCG
Idh3b-F	GGAATATAGCCAACCCCACAG
Idh3b-R	TGTAGCCTCCCATGTCTCG
Idh3g-F	AGTGGGCGTAAGAAAGTGAC

Idh3g-R	CAAAGGTGATCTGAGGGTAGTG
Sdha-F	GATTACTCCAAGCCCATCCAG
Sdha-R	ACAGTCAGCCTCATTCAAGG
Sdhc-F	GTCTCTTCCTATGGCACTGTC
Sdhc-R	CAAACACAGGGACTTCACAAAC
Sucla2-F	TCCAGCAAGTAACAGAAGCG
Sucla2-R	ACTGCCATGACTATTCCTTGTG
Fh1-F	ATATTCGCTTCCTGGGTTCTG
Fh1-R	GTCATCGCTTCACACTGAGTAG
Pten-F	TGTAAAGCTGGAAAGGGACG
Pten-R	CCTCTGACTGGGAATTGTGAC
Akt1-F	GCCCTCAAGTACTCATTCCAG
Akt1-R	ACACAATCTCCGCACCATAG
Akt2-F	TTACAGCCCTCAAGTATGCC
Akt2-R	GACACAATCTCTGCTCCATAAAAG
Rptor-F	CACTCCTTGTCTTCATCTGGG
Rptor-R	TGTCATGGTCCTATGTTCAGC
Rictor-F	ATGGAAATAAGGCGAGGTCTG
Rictor-R	AAAGCCTCCAACTGTCCTG
Atp5o-F	TCATCTCTGCCTTTTCCACC
Atp5o-R	TGGTTTGGACTCAGGAAGC
Cox7a1-F	TGAGGACGCAAAATGAGGG
Cox7a1-R	CTTTCAAGTGTACTGGGAGGTC
Ndufb6-F	CTTCGCTGTTTCTCATGTGC
Ndufb6-R	CCAGTCTCCAGAATTGTATCACC
Uqcrb-F	GACTTACCCAGAAGGCAGC
Uqcrb-R	CATCTCGCATTAACCCCAGT
PGC-1α-F	CACCAAACCCACAGAAAACAG
PGC-1α-R	GGGTCAGAGGAAGAGATAAAGTTG
β-Actin-F	ACCTTCTACAATGAGCTGCG
β-Actin-R	CTGGATGGCTACGTACATGG
Primers for mitochondria DNA quantification	
mt DNA qPCR primer-F	CATTTATTATCGCGGCCCTA
mt DNA qPCR primer-R	TGTTGGGTTGTTTGATCCTG
β-globin gDNA qPCR -F	GAAGCGATTCTAGGGAGCAG
β-globin gDNA	GGAGCAGCGATTCTGAGTAGA

qPCR -R	
Primers for ChIP-PCR	
Akt1-P1 region-F	GAGCATCCGAGTGAGAAG
Akt1-P1 region-R	CTCCTCCTCTCCTACTTCC
Akt1-P2 region-F	AGTAGGAGAGGAGGAGTTG
Akt1-P2 region-R	CAGCAGCAGTTCTGTTCT
Akt1-P3 region-F	CTGAGAGGAGAGTGAGTTC
Akt1-P3 region-R	TTAGTAGTCCACGCTATCG
Akt1-P4 region-F	AGGACTGAGGATGGATAAGA
Akt1-P4 region-R	AAGACCTTGCTGTGGACT
Akt1-P5 region-F	AGTCCACAGCAAGGTCTT
Akt1-P5 region-R	CCACAGCAACTGATAAGGA
Akt2-P1 region-F	GGAACTAGGAAGGCAGGA
Akt2-P1 region-R	CAGTAGGACACCAACAAGTA
Akt2-P2 region-F	TGACGGGTGCCTAAAGTA
Akt2-P2 region-R	CAGCCACAAACAGGAACT
GAPDH ChIP-F	CAAGGAGCCAAGACTAGATT
GAPDH ChIP-R	TCAAGAGCCTATTGCTAAGT
Primers used for subclone	
mIgfbp7 shRNA-1-F	CCGGCCTCCATGAAATACCACTGAACTCGAGTTCAGTGGTATTTCATGGAGGTTTTTG
mIgfbp7 shRNA-1-R	AATTCAAAAACCTCCATGAAATACCACTGAACTCGAGTTCAGTGGTATTTCATGGAGG
mIgfbp7 shRNA-2-F	CCGGCCTCATCTGGAACAAGGTAAACTCGAGTTTACCTTGTTCCAGATGAGGTTTTTG
mIgfbp7 shRNA-2-R	AATTCAAAAACCTCATCTGGAACAAGGTAAACTCGAGTTTACCTTGTTCCAGATGAGG