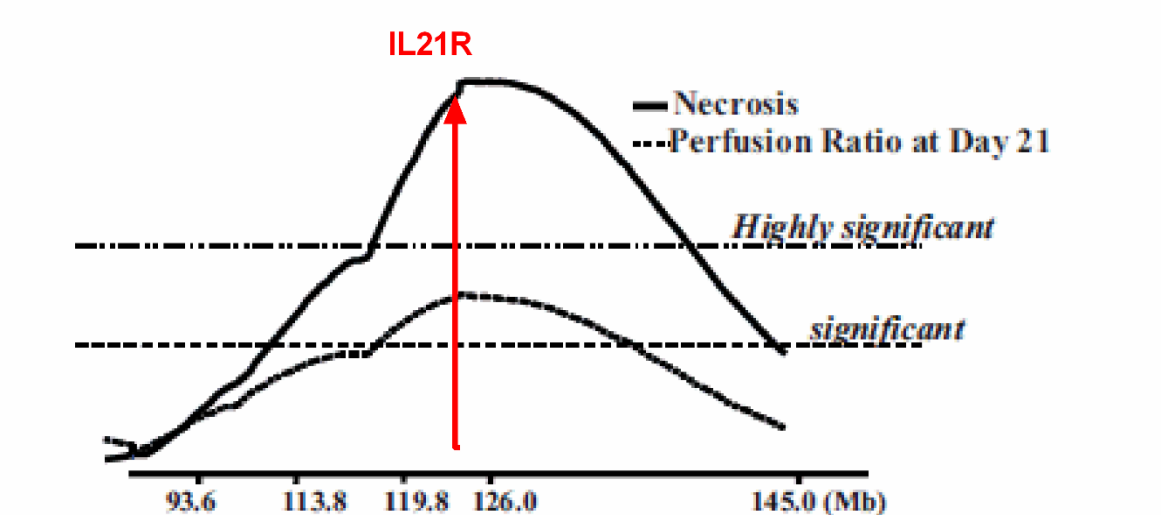
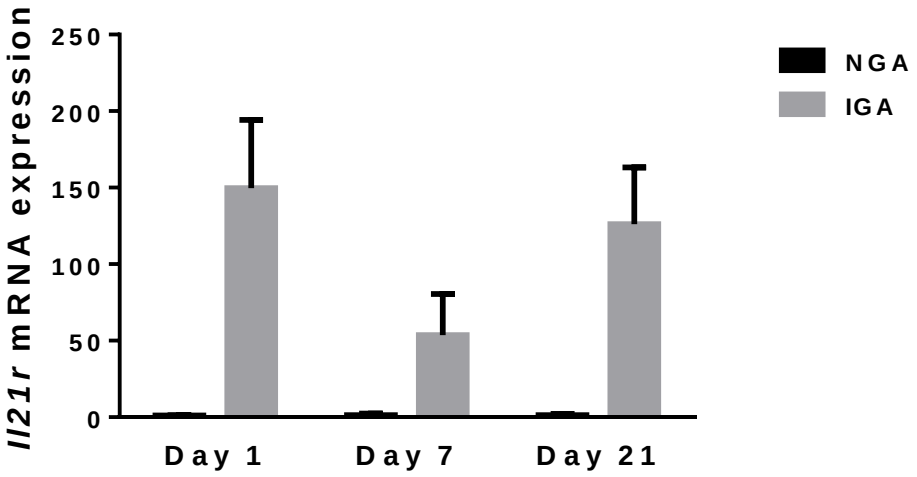
Supplemental Figures.

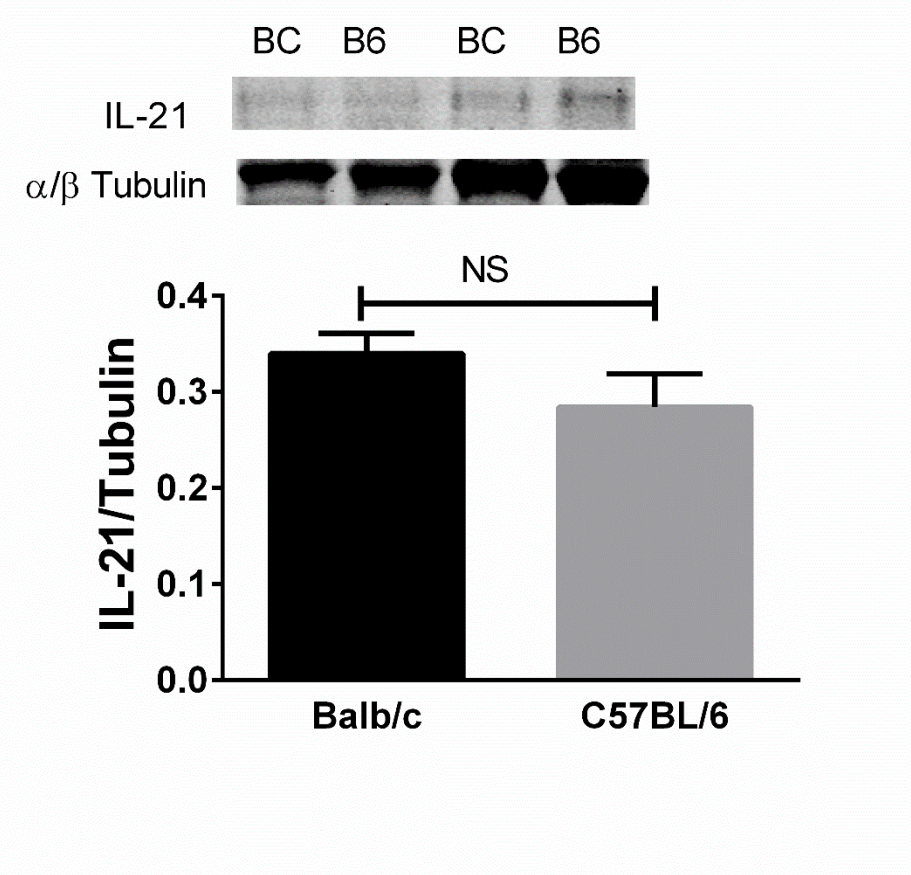
Supplemental Figure I. Modified from Dokun et al (Circ 2008), which shows the boundaries of Lsq-1 with the peaks of effects on necrosis and perfusion recovery. The exact coordinates of IL21R is 125.603252 --- 125.633570, which places the gene at/near the peak of this QTL. Genomic locations are based on the NCBI37/mm10 genome assembly.



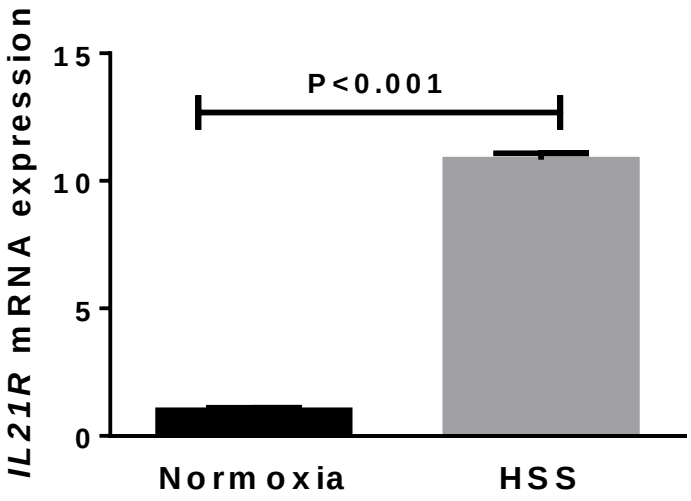
Supplemental Figure II. To investigate whether IL-21R elevation after HLI in the time point other than 3 day, we checked the expression of IL-21R in ischemic and non-ischemic hindlimb muscle from C57BL/6 mice 1, 7, and 21 days after HLI. At each time point, IL-21R level was higher in the ischemic side than non-ischemic side. IGA indicates ischemic gastrocnemius muscle, NGA indicates non-ischemic gastrocnemius muscle. N=4~7/group. Data represent mean ± SEM.



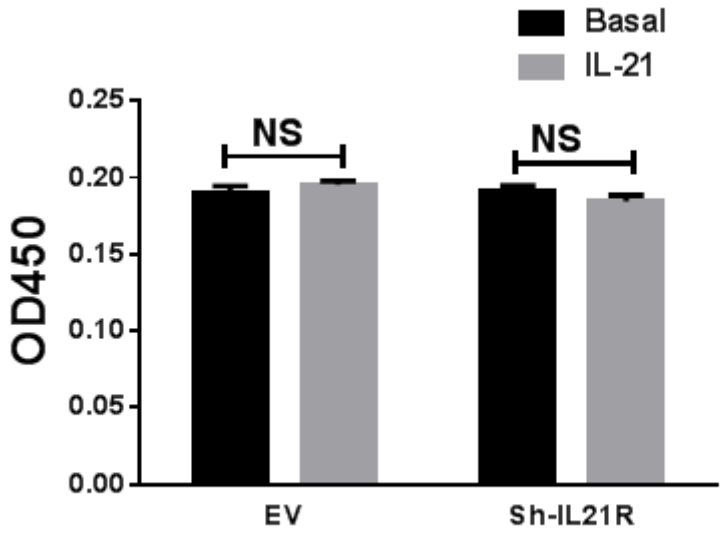
Supplemental Figure III. IL-21 level in ischemic hindlimb muscle 3 days after HLI is not statistically different between Balb/c mice and C57BL/6 mice**.** BC, BALB/c mice; B6, C57BL/6 mice; IGA, ischemic gastrocnemius muscle; NGA, nonis-chemic gastrocnemius muscle. N=6/group. Data represent mean ± SEM.



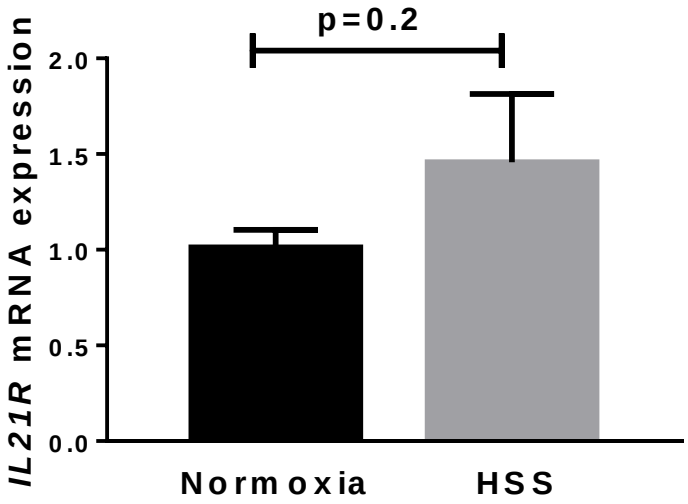
Supplemental Figure IV. *In-vitro* knockdown of IL-21R in HUVECs. HUVECs were transfected with 1 µg/well (6 well dishes) of plasmid vector delivering shRNA targeting IL-21R or nonsense control. 24 hours after transfection, HUVECs were exposed to HSS conditions for an additional of 24 hours. Then *Il21r* mRNA level was measured with qPCR, shRNA knocked down expression of *Il21r* mRNA by 75 ± 13%. Data represent mean ± SEM. Data are representative of three experiments.



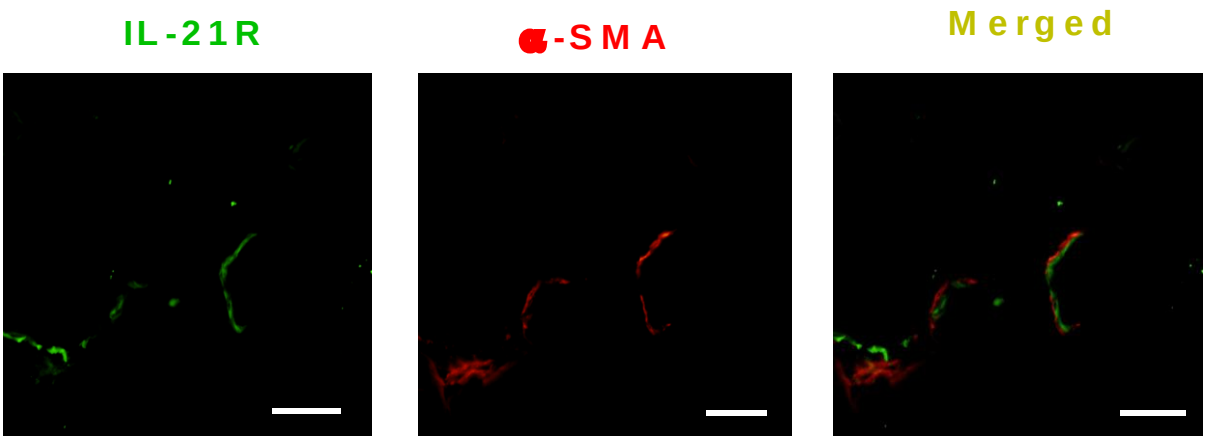
Supplemental Figure V. Under normoxia condition, IL-21 treatment did not change the viability of HUVECs.



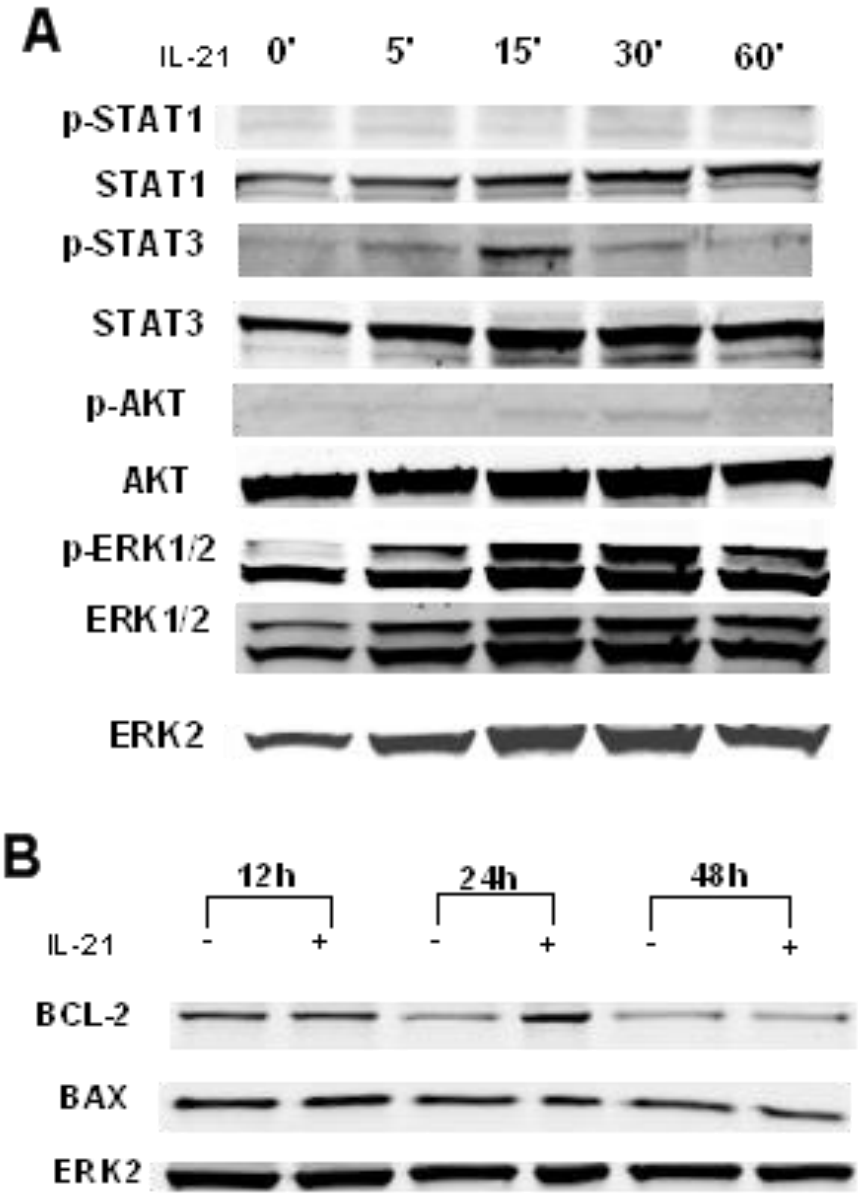
Supplemental Figure VI. Expression of IL-21R mRNA was quantitated using qPCR in an immortalized myocyte cell line (C2C12) under normoxic and HSS conditions. Relative expression of IL-21R in C2C12 cells was not statistically different between these conditions. Data represent mean ± SEM; representative data from three different experiments using different passages of C2C12 cells.



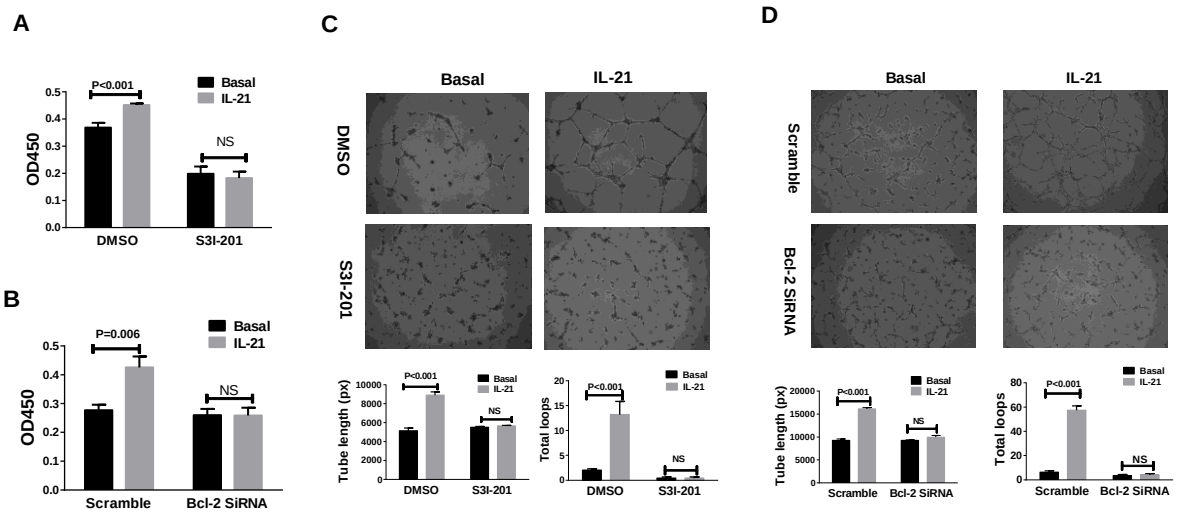
Supplemental Figure VII. Immunofluorescence of ischemic gastrocnemius muscle, IL-21R (green), α-SMA(red) and merged from Wild Type (WT) C57BL/6 mice; co-staining of α-SMA and IL21R cannot be visualized in the ischemic muscle.



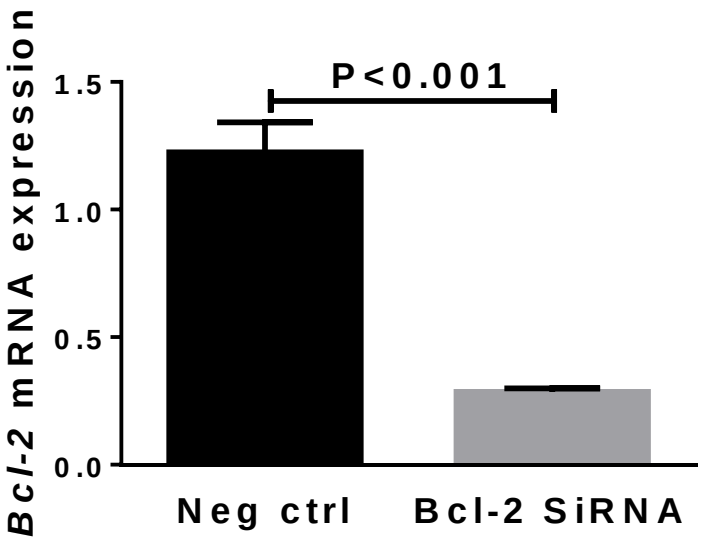
Supplemental Figure VIII. IL-21 affects STAT3 phosphorylation and BCL-2 BAX ratio in a time-dependent manner. (A) After exposed to HSS condition for 24h to induce IL-21R upregulation, HUVECs were treated with IL-21 for 5, 15, 30 and 60 minutes. Cell lysate were collected to measure STAT1, STAT3, AKT and ERK1/2 phophorylation by Western Analysis. STAT3 showed a peak phosphorylation 15 minutes after IL-21 treatment; however, the other protein did not show a significant phosphorylation at any of the selected time point. (B) IL-21 stimulated BCL-2/BAX ratio increase at a time-dependent manner. HUVECs were treated with/without IL-21 for indicated time point, and showed a peak increase of BCL-2/BAX ratio compared to cells with IL-21 treatment for 24h.



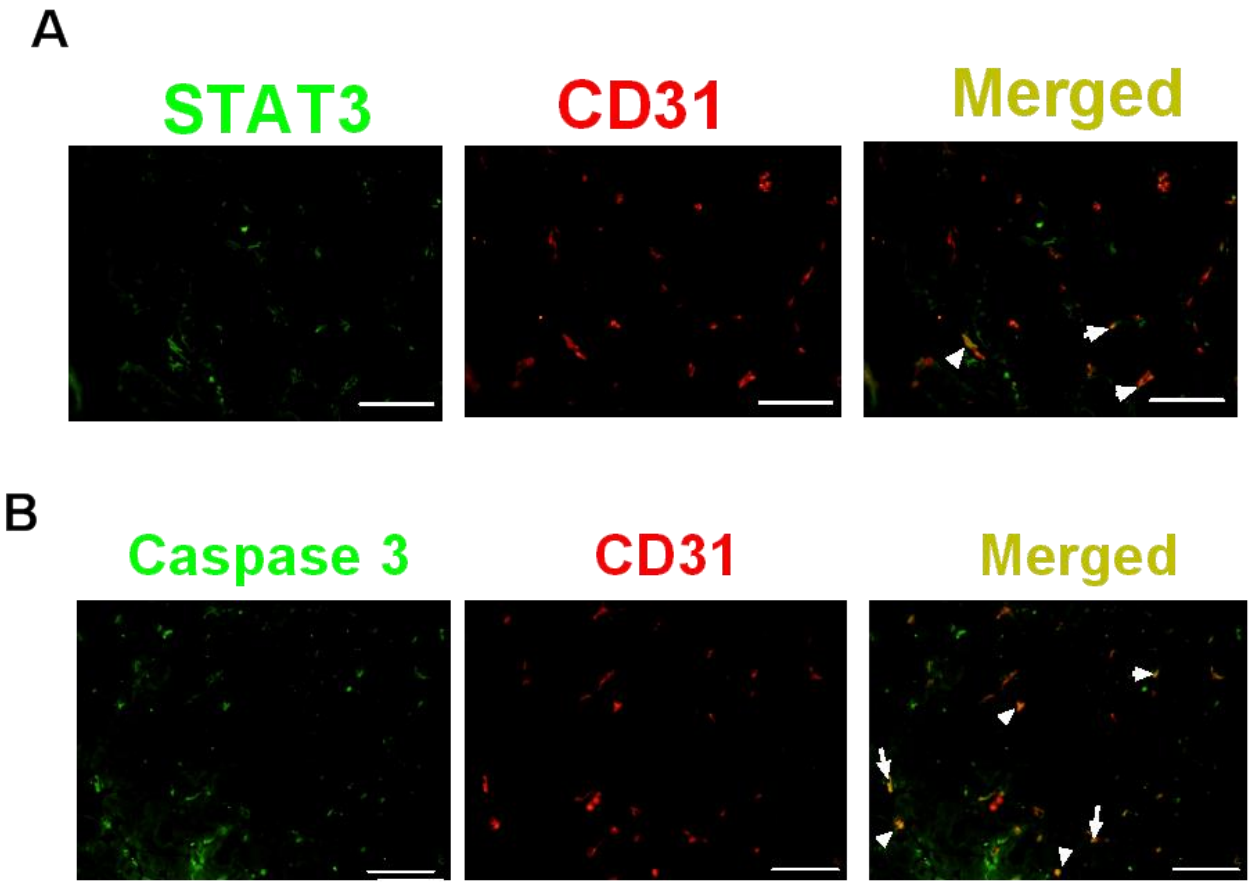
Supplemental Figure IX. STAT3 inhibitor (S3I-201) blunted IL-21 induced cell viability (A) and tube formation (C) increase. When BCL-2 expression were knocked-down by SiRNA, IL-21 treatment did not show significant increase of cell viability (B) or tube formation (D) for HUVECs under HSS condition.



Supplemental Figure X. *In-vitro* knockdown of BCL-2 in HUVECs. HUVEC were transfected with BCL-2 SiRNA or its negative control. 24h after transfection, *bcl-2* mRNA level were quantified by qPCR, which showed that *bcl-2* mRNA were knocked down ~70% by SiRNA. Data represent mean ± SEM.



Supplemental Figure XI. Immunofluorescence of ischemic gastrocnemius muscle from C57BL/6 mouse. (A) Co-localization of STAT3 (green) and CD31 (red) can be found, as pointed by arrows. (B) Co-localization of Caspase 3 (green) and CD31 (red) can be found, as shown with arrows.



Supplemental Figure XII. STAT3 phosphorylation and BCL-2/BAX in ischemic and non-ischemic gastrocnemius muscle after HLI. (A) To determine whether STAT3 is activated after HLI, protein was isolated from ischemic and non-ischemic gastrocnemius muscle 1 day after HLI for western blotting of p-STAT3 and STAT3. The p-STAT3/STAT3 ratio was used to represent the degree of STAT3 activation, which showed that ischemic muscle has significantly higher STAT3 activation (p=0.02). n = 3/group, Data represent mean ±SEM. (B) BCL-2/BAX ratio is decreased in the ischemic gastrocnemius muscle after HLI when compared to non-ischemic gastrocnemius muscle. NGA = non-ischemic gastrocnemius muscle, IGA = ischemic gastrocnemius muscle.