SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Animal models

Fyn Transgenic Mice: Murine FynB and FynT cDNA was generated from 3T3-L1 adipocytes and mouse spleen RNA respectively, using the SuperScript First-Strand Synthesis System (Invitrogen) for reverse transcriptase-polymerase chain reaction (RT-PCR) with the following oligonucleotides: 5'-CACCATGGGCTGTGTGCAATGTAAGG-3' and 5'-CAGGTTTTCACCGGGCTGAT-3' according to the manufacturer's instructions. The extracted and purified PCR products were cloned into pCRBlunt Ⅱ-TOPO (Invitrogen) and sequenced. 1.5-kilobase fragments of either the FynB or FynT cDNA were ligated into the transgenic vector pStec-HSA, which contains a human skeletal muscle actin (HSA) promoter with the SV40 polyadenylation and intron sequences. The resulting vector, pStec-HSA-FynB or pStec-HSA-FynT were linearized with Cla Ⅰ and microinjected into the nucleus of pre-implantation embryos from C57BL6/129svj background at Maine Medical Center Research Institute Core Facility. The resulting pups were screened for the presence of the transgene by PCR of genomic DNA and the positive animals bred onto the C57BL6/129svj background.

Fyn Knockout Mice: Eight to ten week old males C57BLK6/J, pp59fyn null mice and their controls were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed as described previously (Yamada et al., 2010). All studies were approved by and performed in compliance with the guidelines of the Yeshiva University Institutional Animal Care and Use Committee (IACUC).

Antibodies and reagents - Rabbit polyclonal antibody against Fyn and PGC1α and mouse monoclonal antibodies against LKB1 and Bcl2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal antibodies against Tubulin and GAPDH were from Abcam (Cambridge, MA, USA). β-actin and Flag mouse monoclonal antibodies, Vps34 and UVRAG rabbit polyclonal antibodies were from Sigma (St. Louis, MO, USA). LC3 rabbit polyclonal antibody and ubiquitin mouse monoclonal antibody were from Novus Biotechnology

Inc (Littleton, CO, USA). Guinea pig polyclonal p62 antibody was from Progen Biotechnik GmbH (Heidelberg, Deutschland). The Atg14 rabbit polyclonal antibody and V5 mouse monoclonal antibody were from MBL international Corp. (Woburn, MA, USA). Beclin1, pS70-Bcl2, pY705-STAT3, STAT3, pT172-AMPKα, AMPKα, pS792-Raptor, Raptor, pT389-p70S6K1, p70S6K1, Bcl-xL, pT307-Akt, pS473-Akt, Akt, ACC rabbit polyclonal antibodies were all from Cell signaling (Boston, MA, USA). pY261-LKB1 antibody was produced by Pacific Immunology Co (Ramona, CA, USA). Leupeptin was from Fisher Bioreagents (Pittsburgh, PA, USA). All other reagents were from Sigma. pRC/CMV-Flag-STAT3-Y705F from Jim Darnell were obtained from Addgene Inc (Plasmid# 8709) (Cambridge, MA, USA). pRC/CMV-myc-hvps34 were kindly gifted by Dr. Jonathan M. Backer.

Quantitative PCR analysis - Frozen muscle samples were placed into QIAzol Lysis Reagent (Quiagen, Valencia, CA, USA), and homogenized using the Bullet Blender (Next Advance Inc, Averill Park, NY, USA) according to the manufacturer's protocol. Total RNA was isolated using RNeasy® Mini Kit accompanied with RNase-Free DNAse Set (Qiagen) and then reverse-transcribed to cDNA using the SuperScript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA). SYBR green (Qiagen) quantitative RT-PCR was performed for measurement of FynB and FynT. Relative expression levels of the mRNAs were determined using standard curves. Samples were adjusted for total mRNA content by comparison with cyclophilin A. The following primers were used; FynB (forward; 5'-GGCCCAGTTTGAAACACTTC-3', reverse; 5'-TCCATACTTCCCCAAACTGC-3'), FynT (forward; 5'-GGCCCAGTTTGAAACACTTC-3', reverse; 5'-TCCAGAAACAACGAGTCACG-3'), cyclophilin A (forward; 5'-GCATACAGGTCCTGGCATCT-3', reverse; 5'-TTACAGGACATTGCGAGCAG-3'). Atrogin1, MuRF1, Bnip3, Bnip3l, CathepsinL, Dystrophin, PGC1α and ACC1α mRNA was

quantified by TaqMan RT-PCR (Applied Biosystems, Branchburg, NJ, USA). Samples were adjusted for total mRNA content by comparison with GAPDH expression with identical C(T) values between WT and HSA-Fyn mice. Relative expression levels of the mRNAs were determined by 2(-Delta Delta C(T)) method. All primer-probe mixtures were from Applied Biosystems (Branchburg, NJ, USA).

Tissue preparation for Histology - Muscle samples were frozen in liquid nitrogen-cooled isopentane, and transverse serial sections were stained with H&E, Cytochrome C Oxidase, Succinate Dehydrogenase, Non-specific esterase, Acid phosphatase and Gomori Trichrome. Immunofluorescence was also performed using a p62 guinea pig polyclonal antibody followed by Alexa Fluor 594 anti-guinea pig IgG. The samples were mounted on glass slides with Prolong Gold anti-fade reagent with DAPI (Invitrogen, Carlsbad, CA). Cells were imaged using a confocal fluorescence microscope (TCS SP5 confocal; Leica microsystems).

Tissue preparation for electron microscopy - Isolated muscles were fixed in 2.5% glutaraldehyde in 100 mM sodium cacodylate (pH 7.43) and 0.25 M sucrose, and osmium/uranyl stained. Ultrathin sections were viewed on a Jeol 1200EX transmission electron microscope (Jeol Ltd., Akishima, Japan) at 80 kV.

Western Blot Analyses - Tissues were rapidly harvested and freeze-clamped in liquid nitrogen and store at -80^{o}C. Protein preparation and blotting were performed as described below. Tissues were homogenized with a Bullet Blender (Next Advance, NY, USA) in a NP-40 lysis buffer containing protease and phosphatase inhibitors (25 mM HEPES, pH 7.4, 10 % glycerol, 50 mM sodium fluoride, 10 mM sodium phosphate, 137 mM sodium chloride, 1 mM sodium orthovanadate, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml pepstatin, 5 μg/ml leupeptin). Homogenates were centrifuged for 15 min at 13,000xg at 4^{o}C and supernatants were collected.

Protein concentration was determined using a BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL, USA).

Protein samples (40 μg) were separated on 8-15% reducing polyacrylamide gels and electroblotted onto Immobilon-P polyvinylidene difluoride membranes (Biorad, Hercules, CA, USA). Immunoblots were blocked with 5% milk in Tris-buffered saline for 2 h at room temperature and incubated overnight at 4°C with the indicated antibodies in Tris-buffered saline and 0.05% Tween 20 (TBST) containing 1% BSA. Blots were washed in TBST and incubated with horseradish peroxidase-conjugated secondary antibodies (1:30,000) for 30 min at room temperature. Membranes were washed in TBST, and antigen-antibody complexes were visualized by chemiluminescence using an ECL kit (Thermo Fisher Scientific). Alternatively, immunoblots were incubated with IRDye800CW Goat Anti Mouse (H+L) or IRDye680 Goat Anti Rabbit (H+L) secondary antibodies and signal was detected with the Odyssey® Infrared Imaging System (Li-COR Biotechnology, Lincoln, NE).

Immunoprecipitation - Tissues were homogenized in a NP40 buffer. Homogenates were centrifuged for 15 min at 13,000xg at 4°C, and supernatants were collected. Protein concentration was determined with Bio-Rad assay. Lysates (3-4 mg) were incubated with 10 μg of Atg14 rabbit polyclonal antibody (MBL international, USA) for 2 h at 4°C. Fifty ml of TrueBlot™ anti-Rabbit Ig IP Beads (eBioscience, Inc., San Diego, CA) was added and samples were consequently rocked for 60 min at 4°C. Samples were extensively washed three times with NP-40 lysis buffer and were resuspended in 100 μl of Laemmli sample buffer containing 50 mM DTT or 2% β-mercaptoethanol. Samples were heated at 90-100°C for 10 min and centrifuged at 13,000xg for 3 min. Supernatants were collected and loaded on SDS-polyacrylamide gels.

Fyn kinase activity - Muscle tissues were rapidly freeze-clamped in liquid nitrogen and homogenized in a NP40 based buffer. Homogenates were centrifuged for 15 min at 14,000xg at 4^{o}C and supernatants were collected. Protein concentration was determined by BCA method. Whole cell lysates (0.5-1 mg) were incubated with 4 μg of Fyn rabbit polyclonal antibody (Santa Cruz Biotechnology) coupled with Catch and Release columns (Millipore, USA) for 2 h at 4^{o}C. Samples were washed three times with NP-40 lysis buffer and once with the kinase reaction buffer. Immunocomplexes were diluted 2.5 fold and 40 μl samples and 10 μl ATP solution were incubated in an ELISA plate. Measurement of the kinase activity was performed according to the manufacturer's protocol.

Transfection of skeletal muscle in vivo - Mice were anesthetized with isoflurane. The right tibialis anterior was injected with 125 μg of indicated vectors and the left tibialis anterior with the pcDNA empty vector as control. Electroporation (8 shocks) was performed using the S48 Square Pulse Stimulator (Grass Technologies, West Warwick, RI) with the following settings: train rate = 1TPS; train duration = 500ms; pulse rate = 1PPS; duration = 20ms, voltage = 80V. Electroporation was repeated 4 days later. Animals were sacrificed 4 days after the second set of electroporation. Transfection with a control LacZ expression plasmid demonstrated that this protocol resulted in greater than 90% of tibialis anterior muscle fibers histologically positive for β-galactosidase activity. Muscle extracts were then used for immunoblotting and autophagic flow as described above.

Phosphotyrosine proteome screen- Tyrosine PhosphoScan® Proteomics screening was provided by Cell signaling Technology (Boston, MA) as detailed on their website http://www.cellsignal.com/services/tyr_phospho.html. Briefly, gastrocnemius skeletal muscle extracts of Fyn null mice and the HSA-FynT mice were digested with trypsin followed by

immunoprecipitation with the phosphotyrosine PY100 antibody. The immunoprecipitates were then subjected to LC-MS/MS analysis to identify tyrosine-phosphorylated proteins.

SUPPLEMENTARY FIGURE LEGENDS

Figure 1S: A) HEK293T cells were co-transfected with Flag-LKB1 and either FynB-V5 or FynT-V5 cDNA. Cell extracts were immunoblotted for V5 and Flag (upper 2 panels) and in parallel immunoprecipitated with the Flag antibody and immunoblotted with the V5 or Flag antibodies (lower 2 panels). These are representative immunoblots independently performed 3 times. **B)** Relative Fyn kinase activity in gastrocnemuius muscle extracts from WT, HSA-FynB and HSA-FynT mice. This is the average kinase activity from 5 independent determinations each performed in triplicate. **C)** Representative higher magnification image of Hematoxylin & Eosin (H&E) staining of EDL muscles from the HSA-FynT mice. Black arrows indicated nuclear hypertrophy; Green arrows indicated elongated nuclei; Stars indicate myofibril vacuoles; and blue arrows indicate central migration of nuclei.

Figure 2S: Representative images of Cytochrome C Oxidase (Cyto CO), Succinate Dehydrogenase (SDH), Non-Specific Esterase (NS Esterase) and Acid phosphatase (APase) staining for EDL (left) and soleus (right) muscles of WT and HSA-FynT mice.

Figure 3S: Representative transmission electron microscopic images of EDL and soleus muscles from WT and HSA-FynT mice. Large arrows indicate expanded mitochondria, small arrows indicate Z-line disruption and arrowheads indicate expanded sarcoplasmic reticulum.

Figure 4S: A) Representative images of modified Gommori Trichrome staining of EDL and soleus muscles of WT and HSA-FynT mice. **B)** TGFβ1 mRNA expression levels relative to Hrpt1 in EDL and soleus muscles of HSA-FynT mice. *$p<0.005$ vs WT.

Figure 5S: A) Gastrocnemius muscle extracts from WT and HSA-FynT mice were immunoblotted for pT172-AMPKα, pS792-Raptor, pS389-S6K1, total AMPKα, Raptor and S6K1. The muscle extracts were also immunoprecipitated (IP) with an LKB1 antibody and immunoblotted for LKB1 and pY261-LKB1. These are representative immunoblots

independently performed 3 times. **B)** Gastrocnemius muscle extracts from WT and HSA-FynT mice were immunoblotted with an ubiquitin antibody from two independent animals. This is a representative immunoblot independently performed 3 times. **C)** PGC1α and (D) ACC1α mRNA expression levels relative to GAPDH mRNA and protein as internal controls in gastrocnemius muscle from WT and HSA-FynT mice.

Figure 6S: RNA isolated from EDL (upper) and soleus (lower) muscle from WT and HSA-FynT mice were subjected to qRT-PCR for the expression of Atrogin1, MurF1, Bnip3L, Bnip3, CathepsinL and Dystrophin relative to GAPDH levels. These data are the average of 5 independent determinations each performed in triplicate. *$p<0.05$, **$p<0.01$, ***$p<0.005$ vs WT.

Figure 7S: A) WT mice were food restricted for 30 h (starved) and then allowed free access to food for 5 h (refed). The EDL and soleus muscles were isolated and immunobotted for pT308-Akt, pS473Akt, p389S6K1, p172-AMPKα, total Akt, S6K1, AMPKα and GAPDH as a loading control from two independent animals. **B)** Soleus and EDL muscle of WT, HSA-FynT were immunobotted with Fyn, phosphoY705-STAT3, total STAT3 and Vps34 antibodies from two independent animals. GAPDH was used as internal loading control. A light (top panel) and dark (second panel) exposure of the Fyn immunoblot is shown in order to visualize the endogenous Fyn protein.

Figure 1S

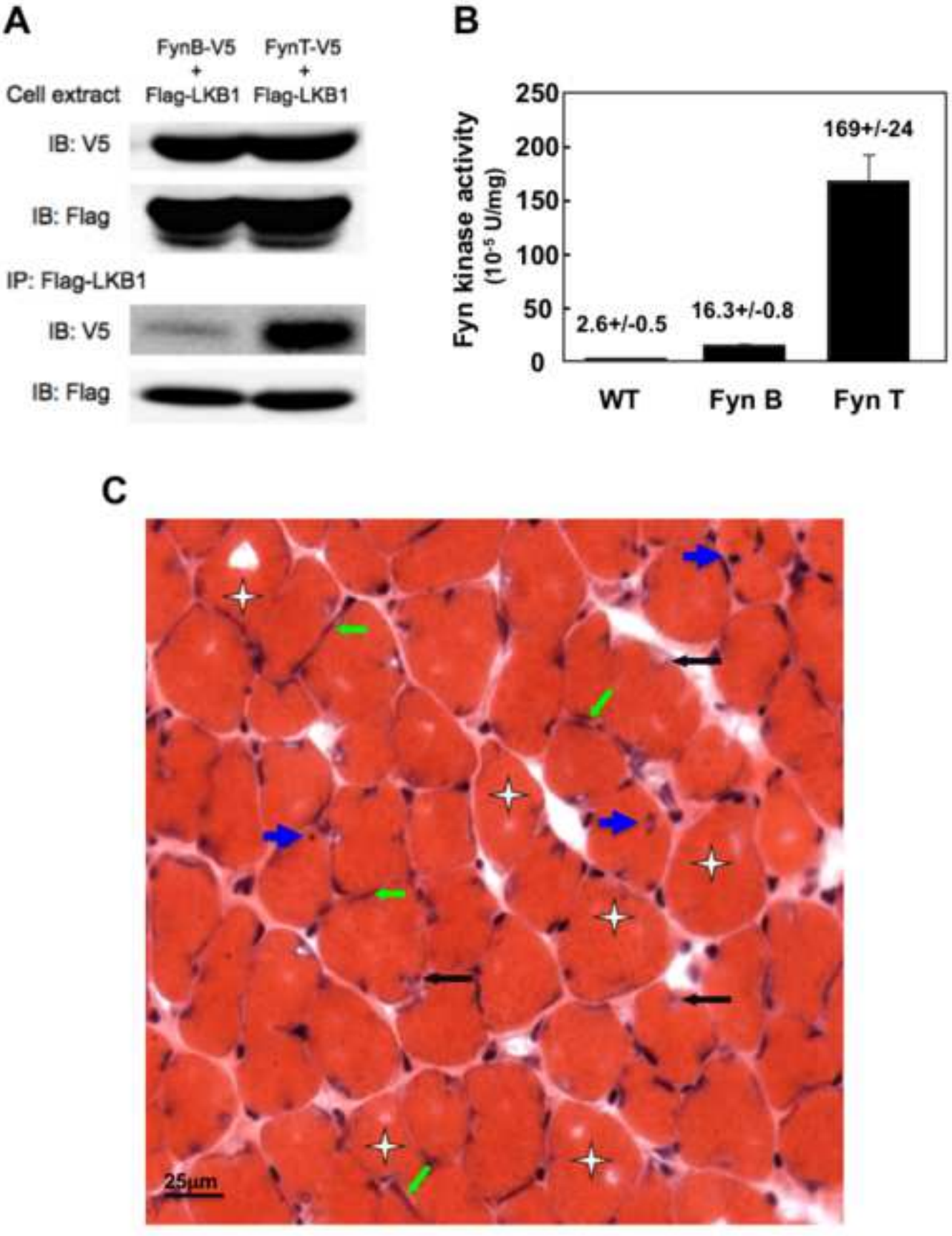

A
FynB-V5 + Flag-LKB1
FynT-V5 + Flag-LKB1
Cell extract
IB: V5
IB: Flag
IP: Flag-LKB1
IB: V5
IB: Flag
B
Fyn kinase activity (10⁻⁵ U/mg)
250
200
150
100
50
0
2.6+/-0.5
16.3+/-0.8
169+/-24
WT
Fyn B
Fyn T
C
25μm

Figure 2S

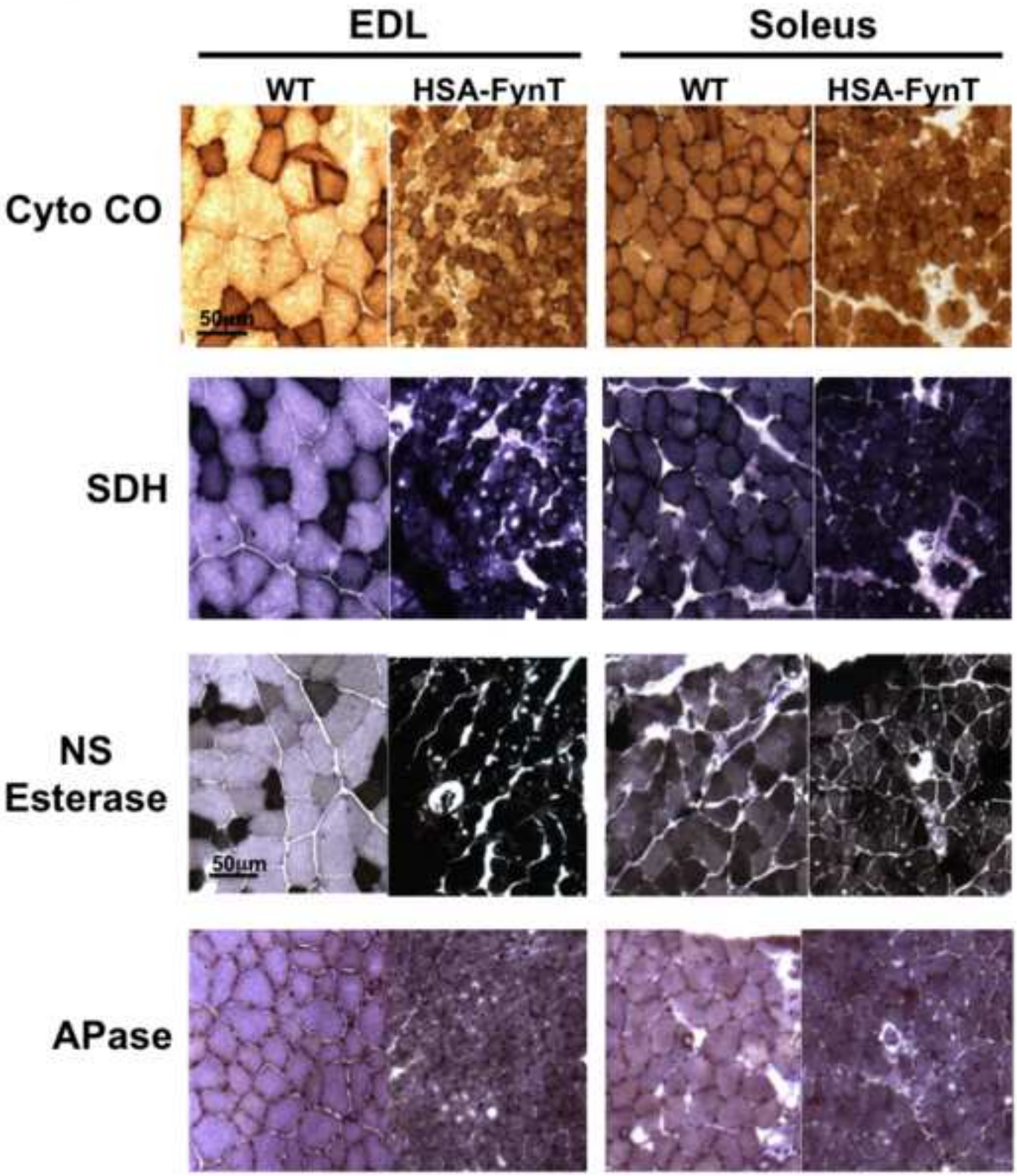


Figure 2S
EDL
Soleus
WT
HSA-FynT
WT
HSA-FynT
Cyto CO
50μm
SDH
NS
Esterase
50μm
APase

Figure 3S

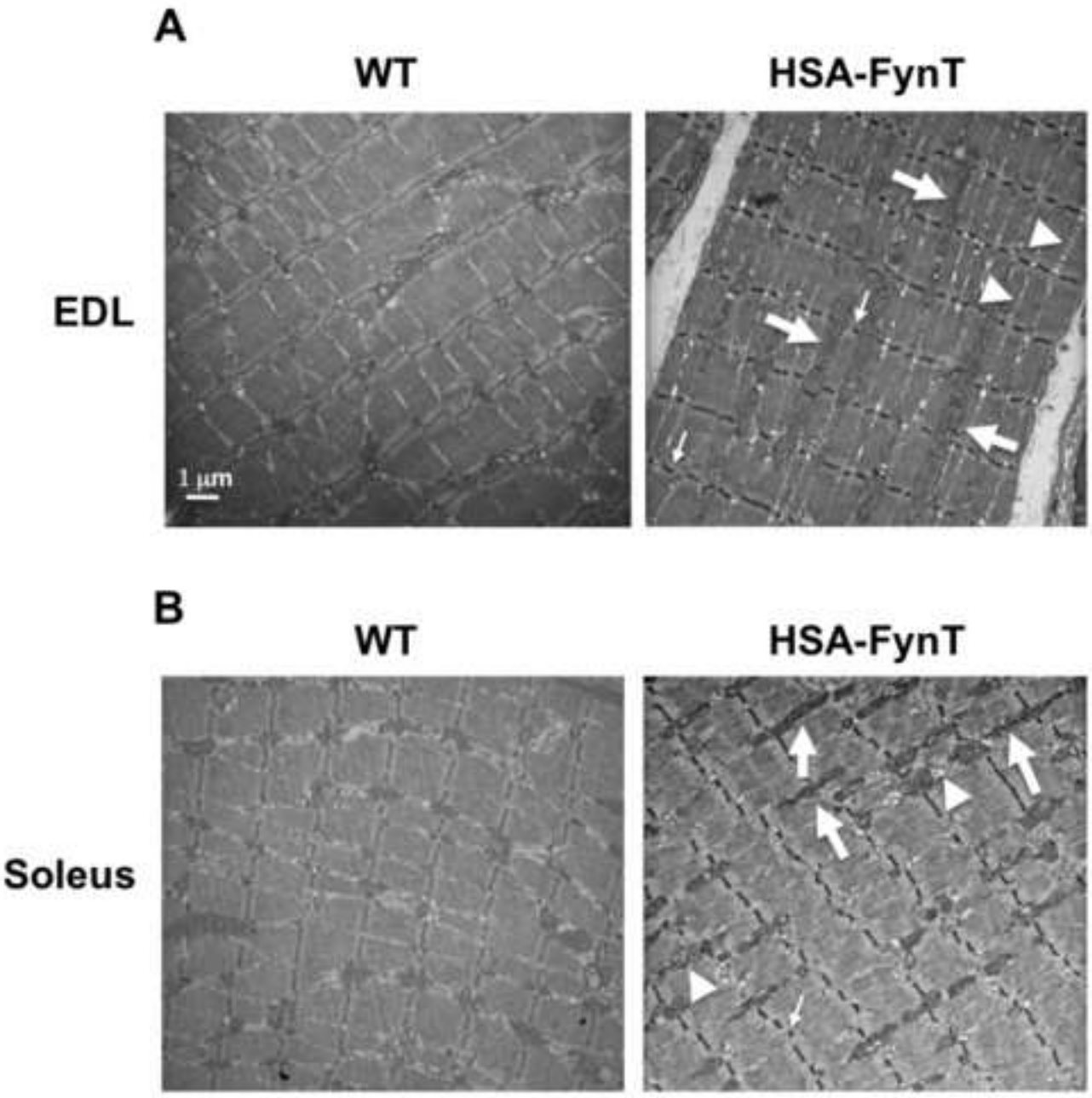
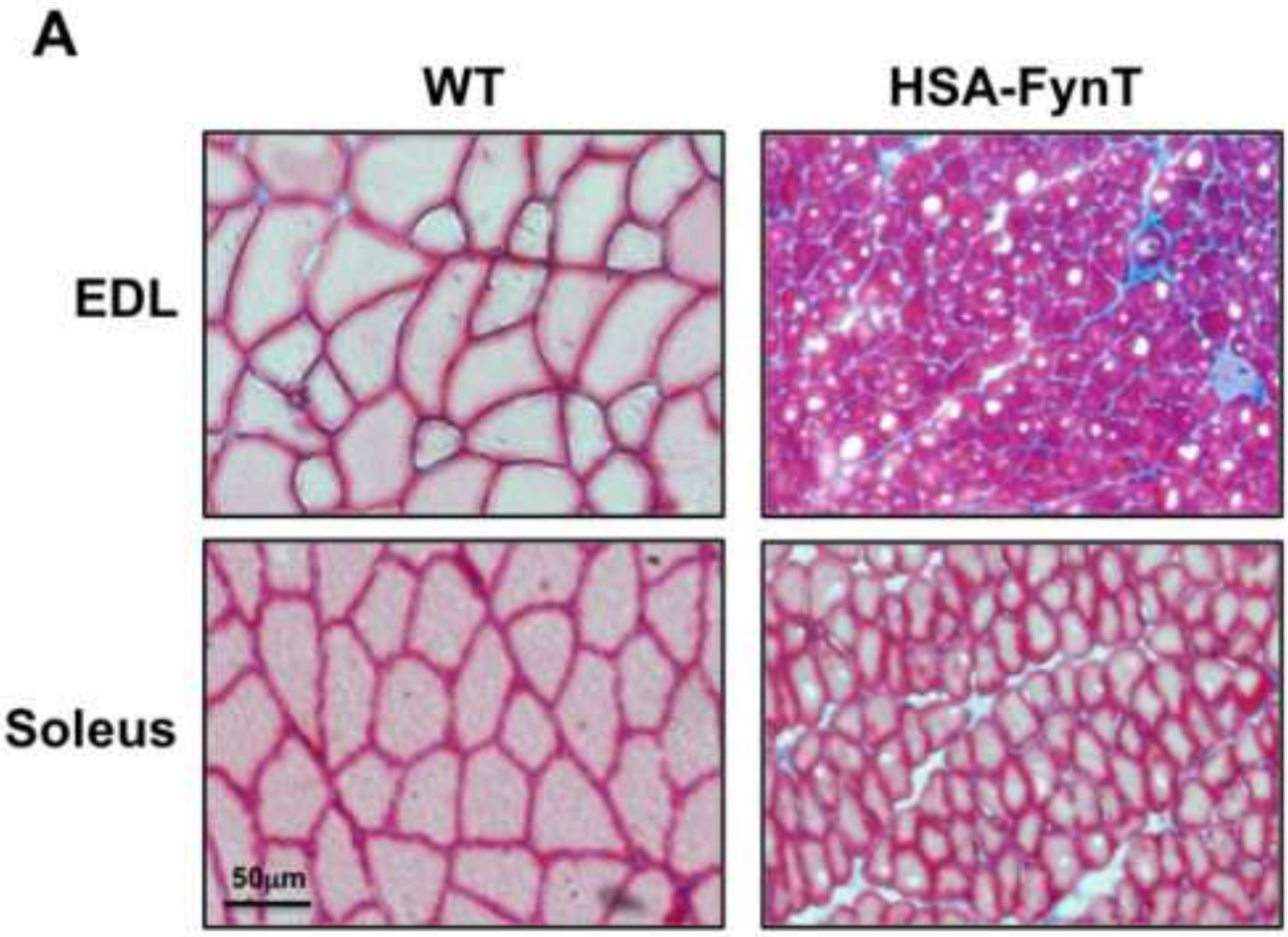


Figure 4S


A
WT
HSA-FynT
EDL
Soleus
50μm

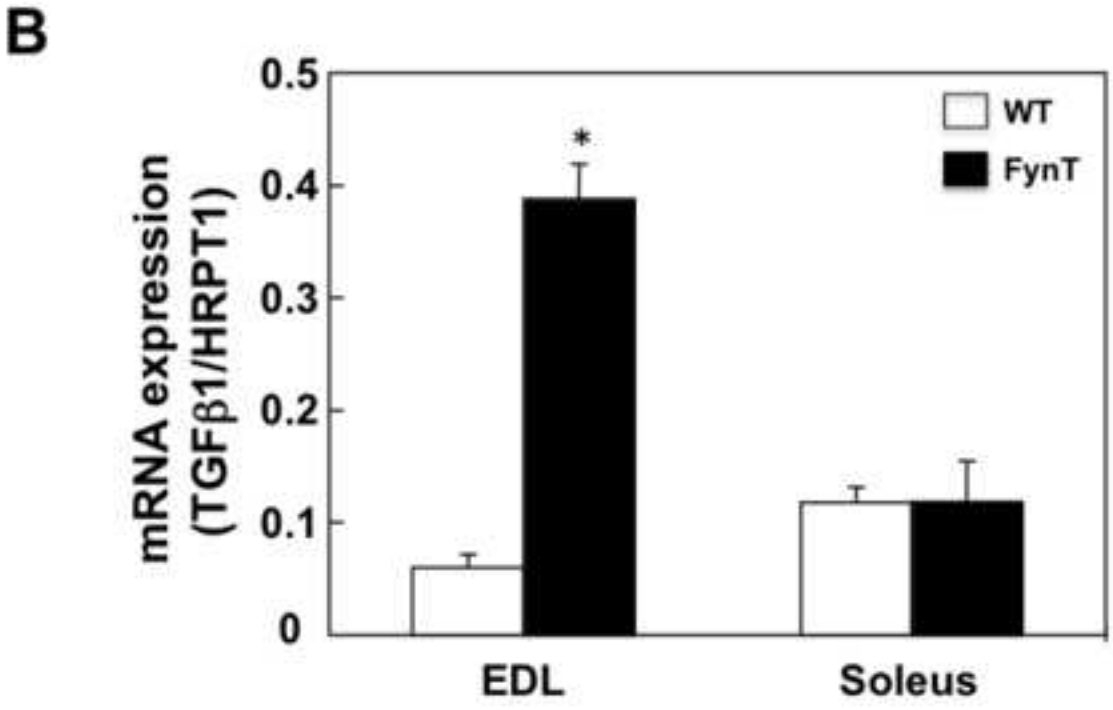

B
mRNA expression
(TGFβ1/HRPT1)
0.5
0.4
0.3
0.2
0.1
0
*
WT
FynT
EDL
Soleus

Figure 5S

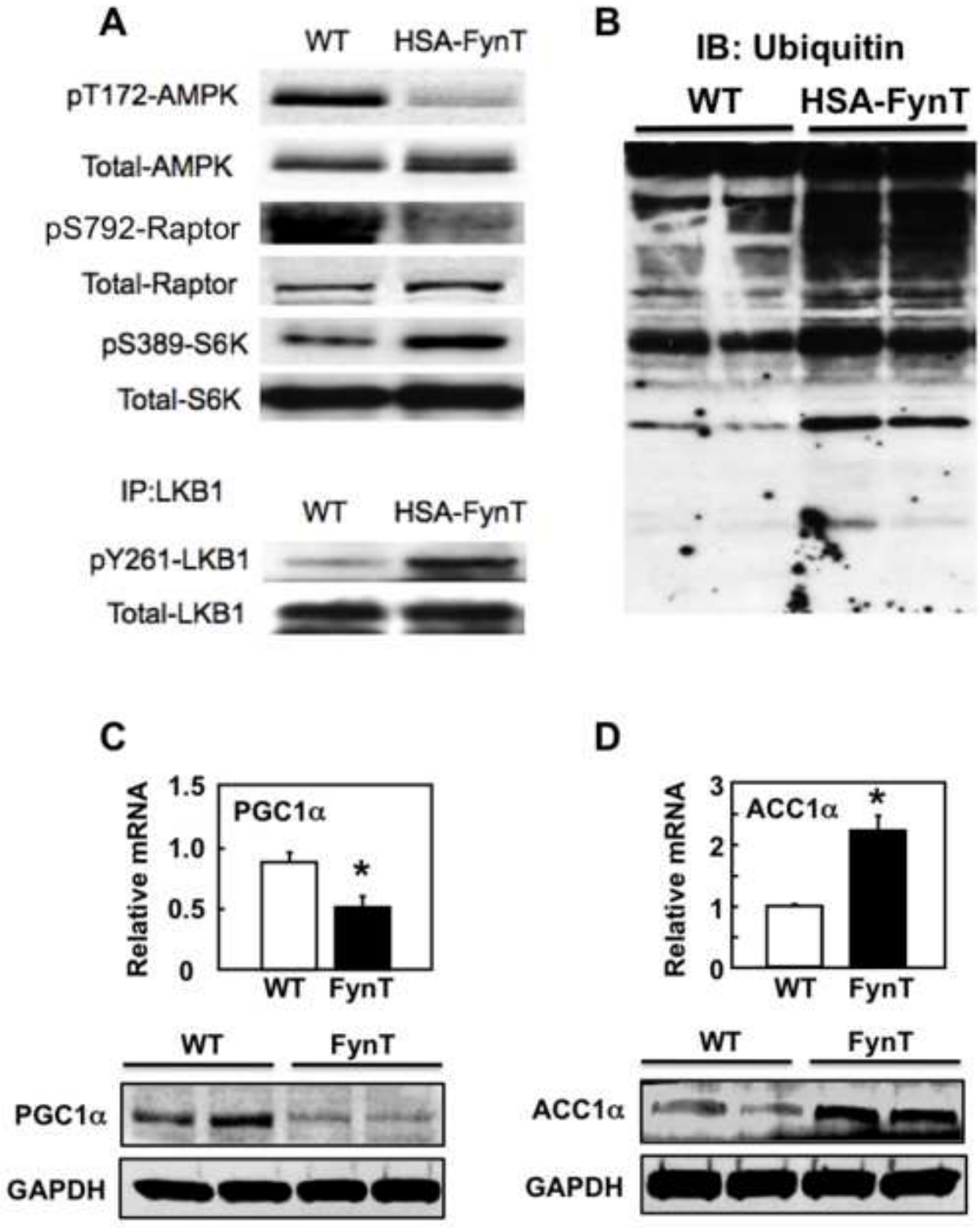

A
WT
HSA-FynT
pT172-AMPK
Total-AMPK
pS792-Raptor
Total-Raptor
pS389-S6K
Total-S6K
IP:LKB1
WT
HSA-FynT
pY261-LKB1
Total-LKB1
B
IB: Ubiquitin
WT
HSA-FynT
C
Relative mRNA
1.5
1.0
0.5
0
PGC1α
*
WT
FynT
WT
FynT
PGC1α
GAPDH
D
Relative mRNA
3
2
1
0
ACC1α
*
WT
FynT
WT
FynT
ACC1α
GAPDH

Figure 6S

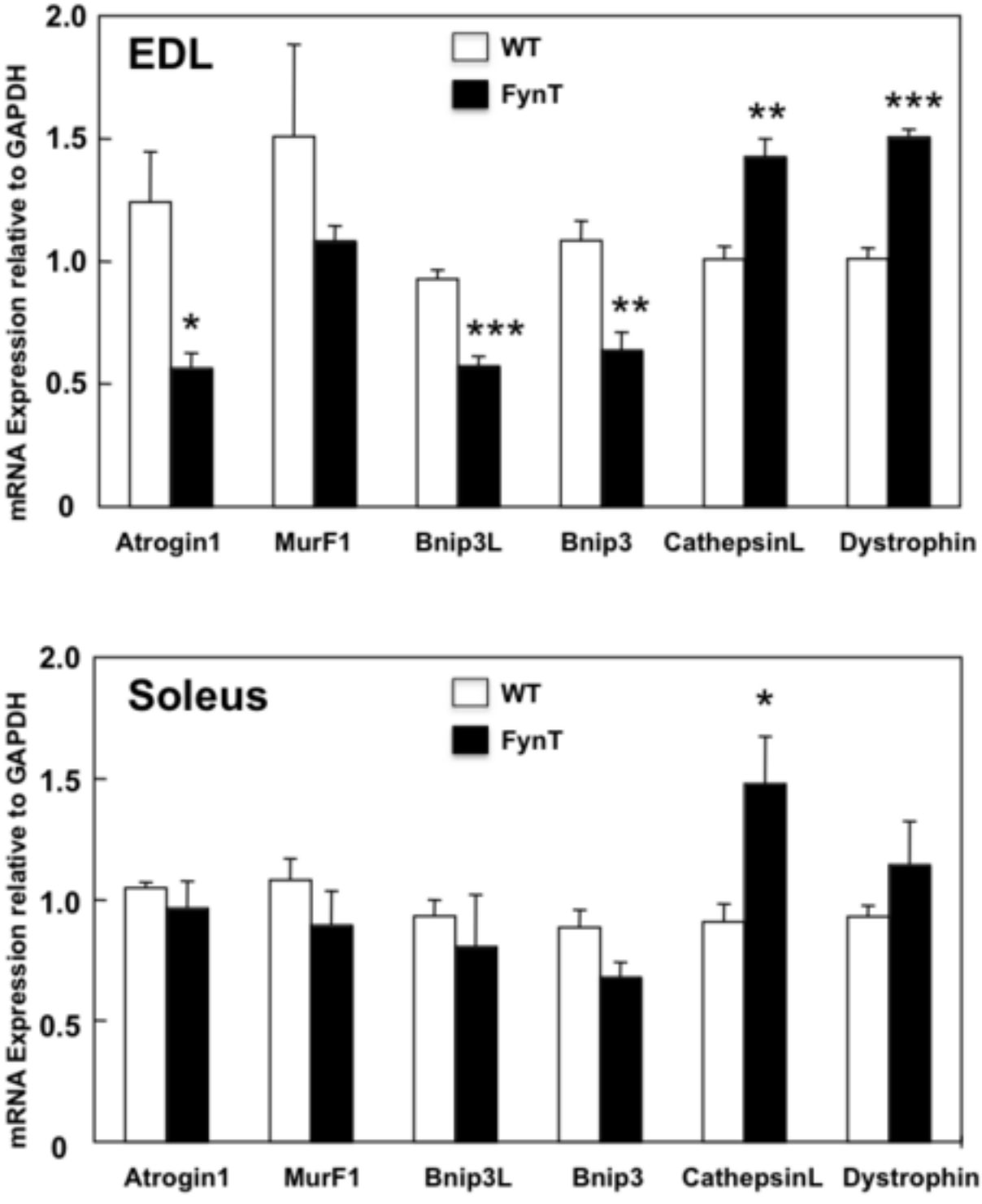
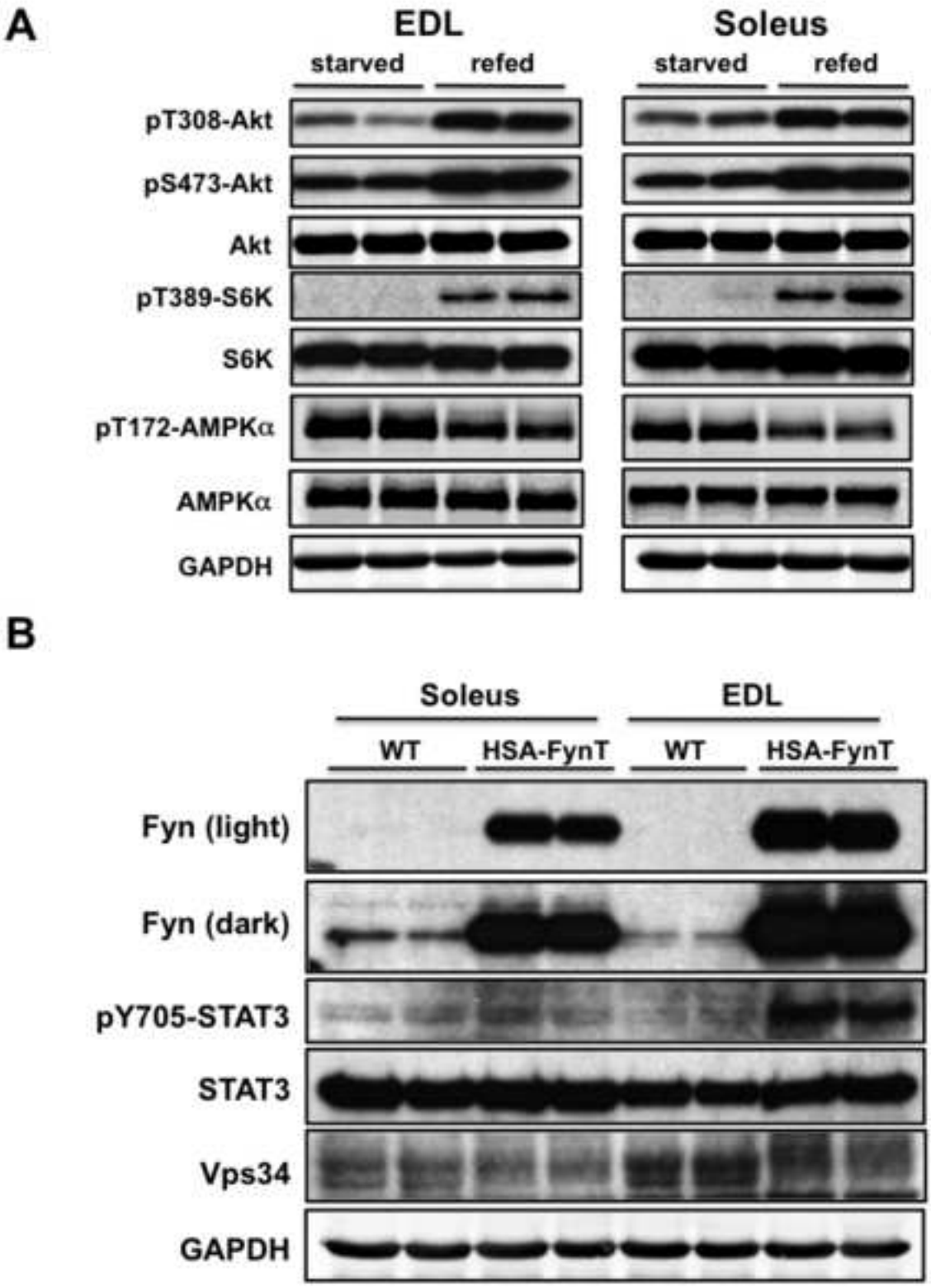

EDL
WT
FynT
mRNA Expression relative to GAPDH
2.0
1.5
1.0
0.5
0
*
***
**
**
***
Atrogin1
MurF1
Bnip3L
Bnip3
CathepsinL
Dystrophin
Soleus
WT
FynT
mRNA Expression relative to GAPDH
2.0
1.5
1.0
0.5
0
*
Atrogin1
MurF1
Bnip3L
Bnip3
CathepsinL
Dystrophin

Figure 7S


A
EDL
Soleus
starved
refed
starved
refed
pT308-Akt
pS473-Akt
Akt
pT389-S6K
S6K
pT172-AMPKα
AMPKα
GAPDH
B
Soleus
EDL
WT
HSA-FynT
WT
HSA-FynT
Fyn (light)
Fyn (dark)
pY705-STAT3
STAT3
Vps34
GAPDH