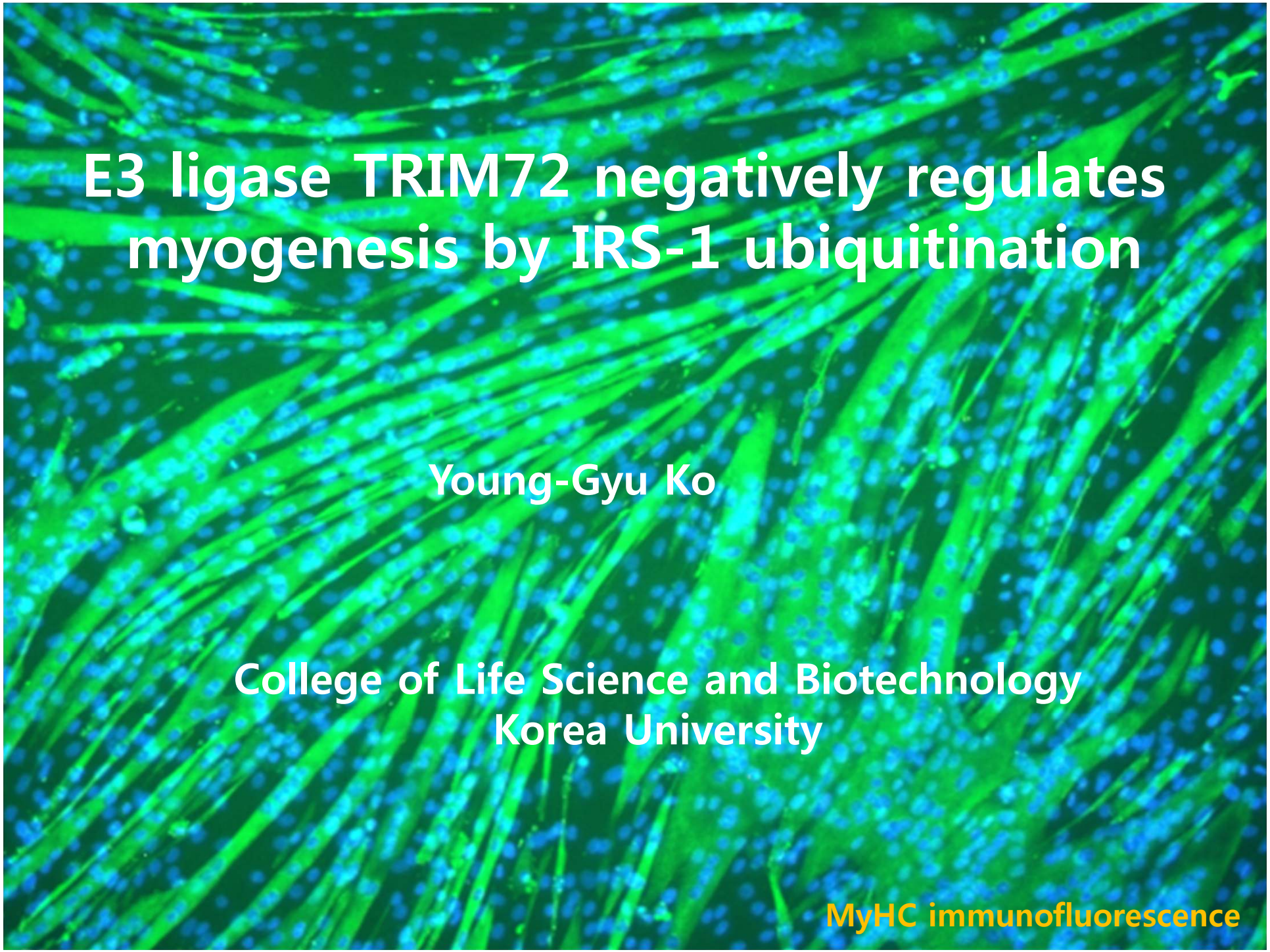
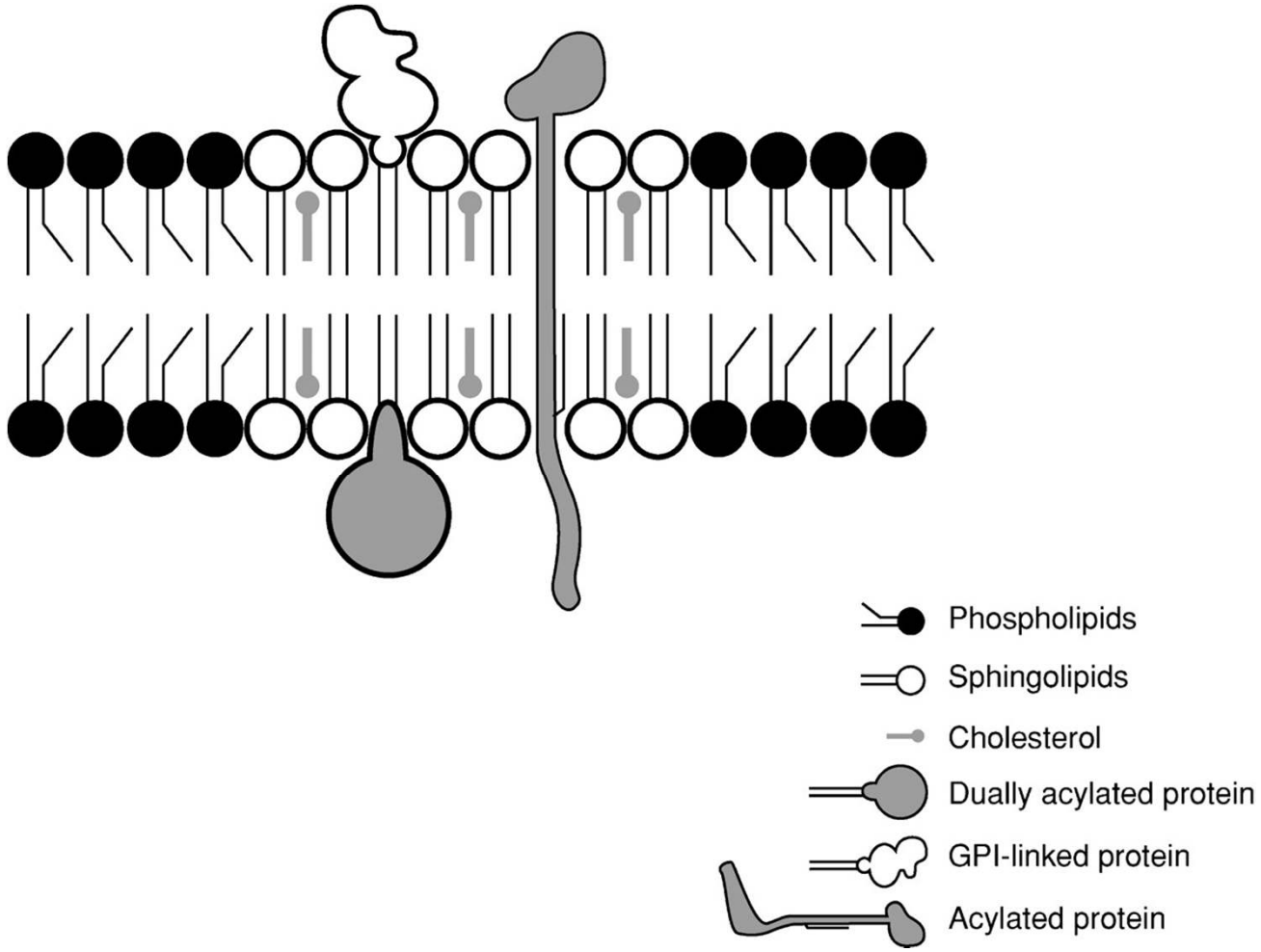
E3 ligase TRIM72 negatively regulates myogenesis by IRS-1 ubiquitination

Young-Gyu Ko

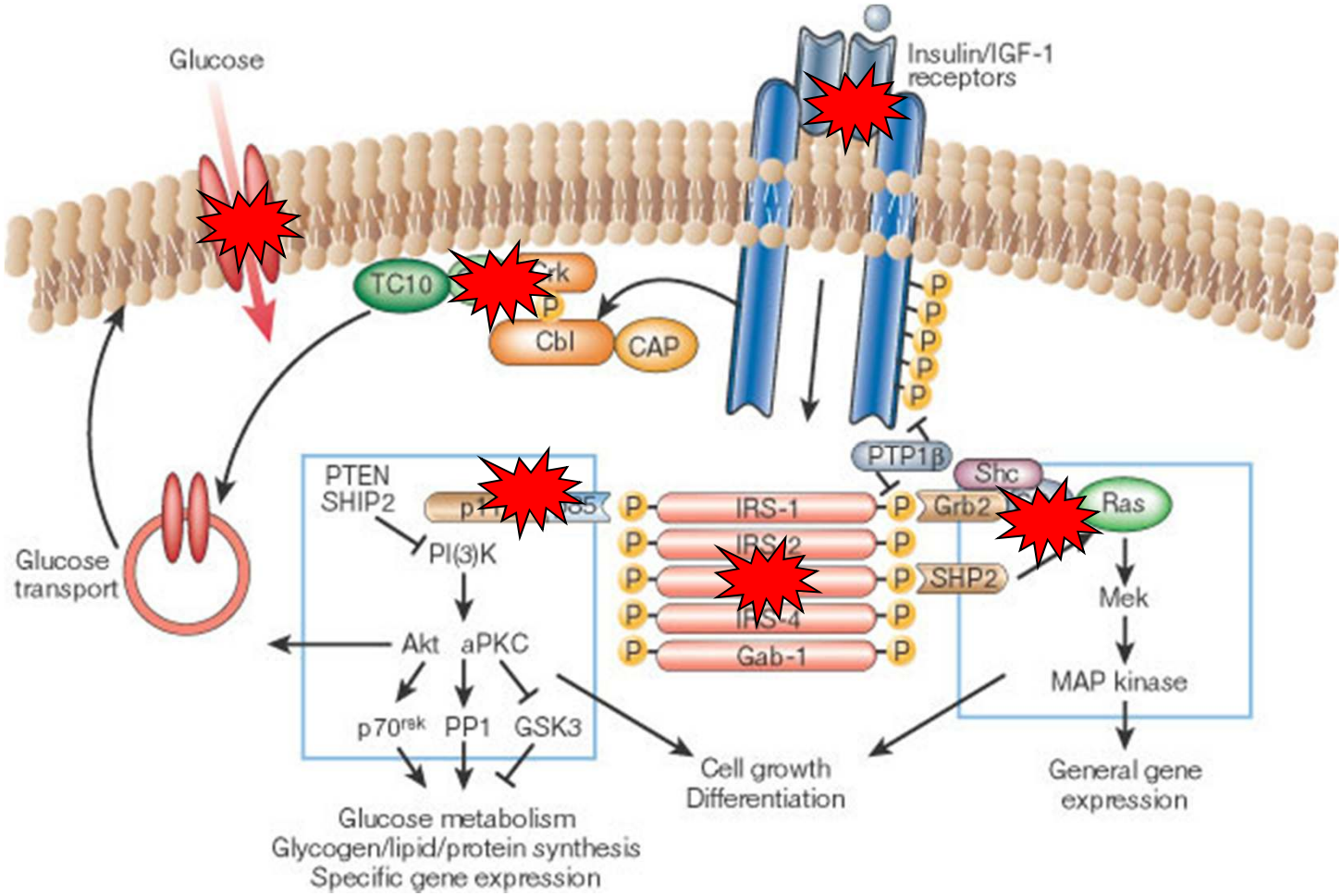
College of Life Science and Biotechnology
Korea University



Lipid rafts are plasma membrane compartments composed of cholesterol and glycosphingolipids.

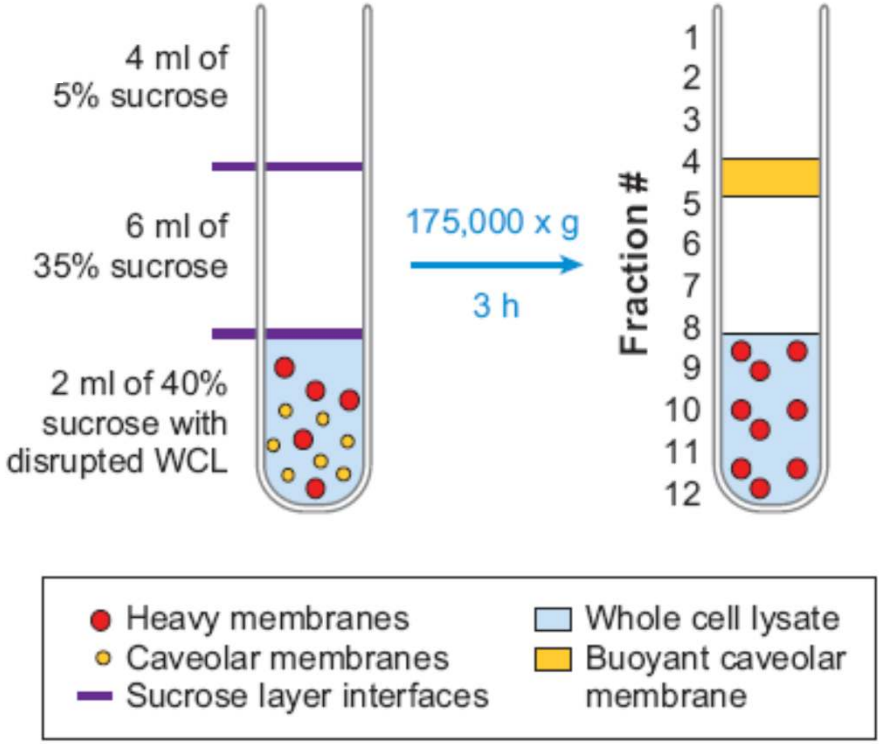


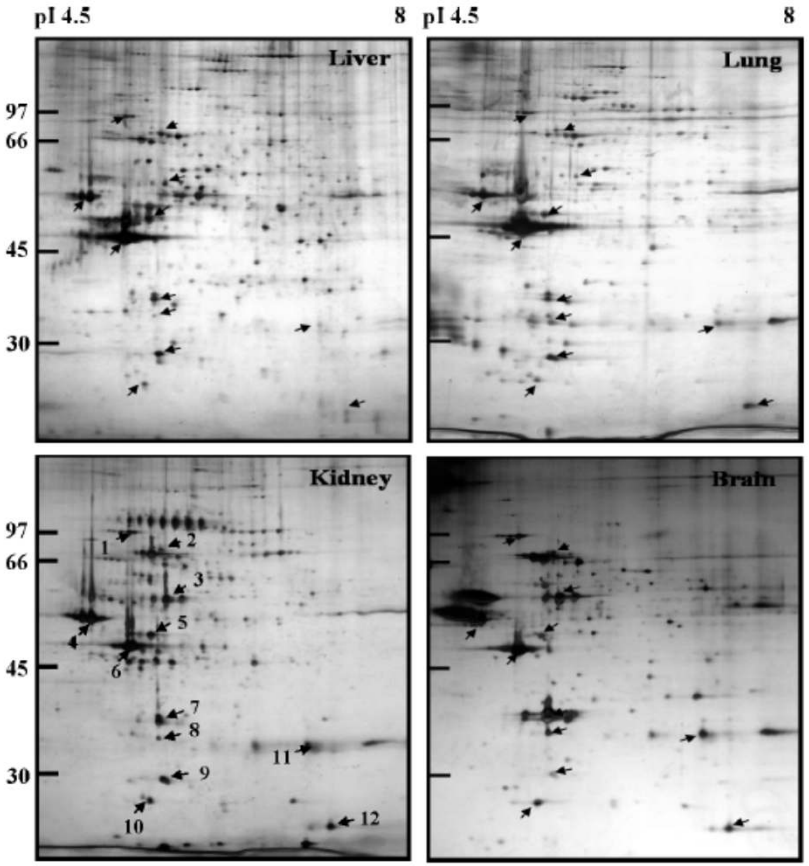
Insulin action through lipid rafts



Signaling molecules found in lipid rafts; IR, IRS, Grb2, Sos, Ras, PI-3-K, TC10, Cbl, CAP, and GLUT4

Identification of novel signaling molecules by lipid raft proteome

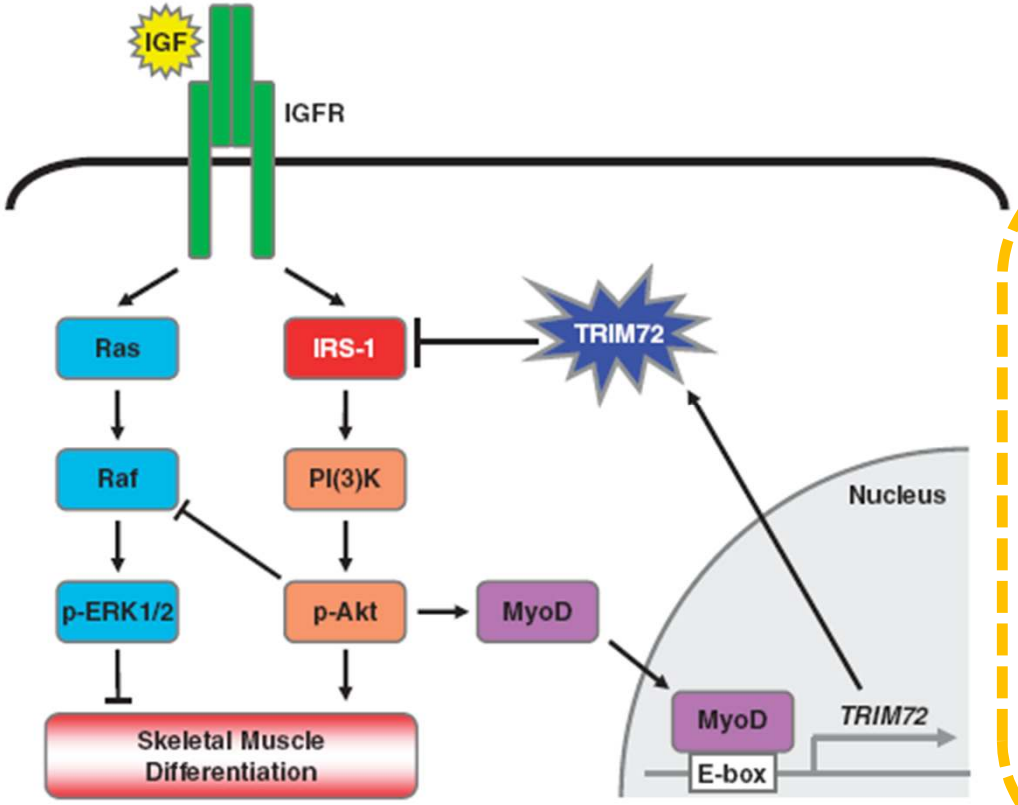




지질래프트는 신호전달물질을 농축하고 있어서 새로운 신호전달물질의 동정에 매우 유리

Functional proteomics of lipid rafts

Functional proteomics of lipid rafts

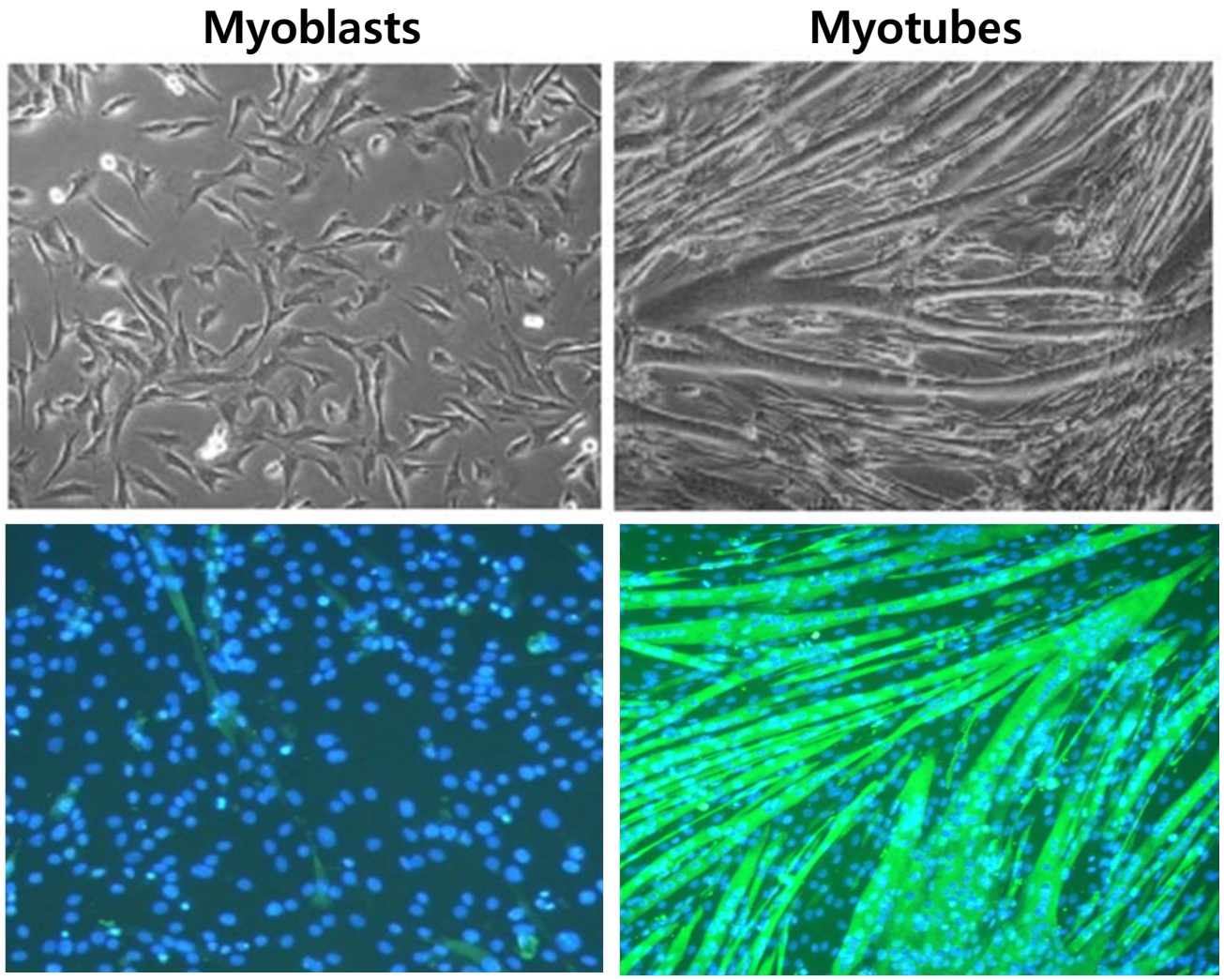


The function of TRIM72
Cell Death Differ, 2010

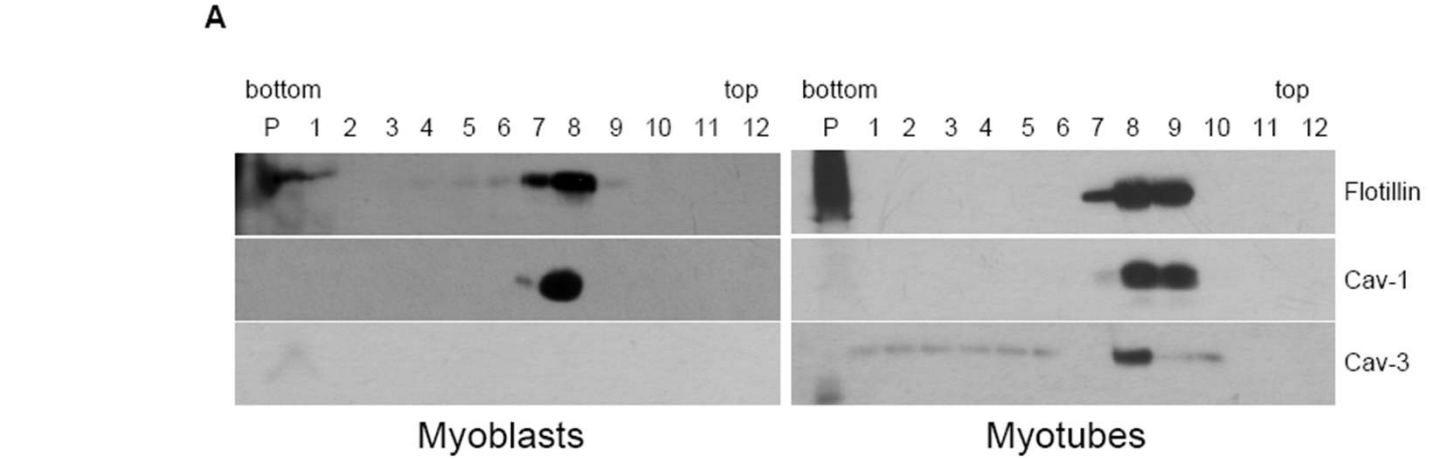
Lipid raft proteome papers in our lab

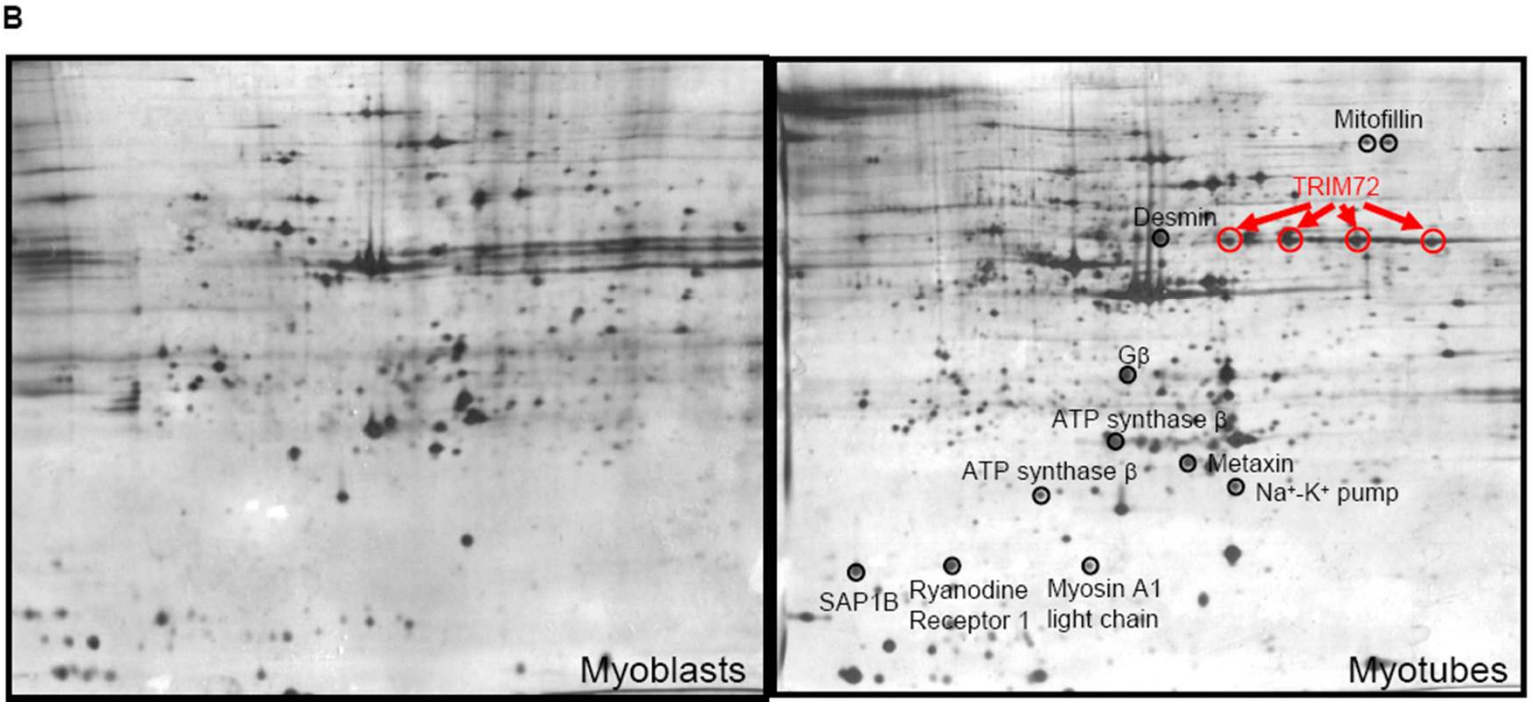
TRIM72, gC1qR, surface OXPHOS
JBC, 2011; a paper of the week
Expert Rev. Proteomics, 2010
CDD, 2010
BBRC, 2010
Proteomics, 2010; cover paper
Proteomics, 2009; issue paper
Proteomics, 2006; cover paper, 51 citations
Diabetologia, 2006; 82 citations
EMM, 2004; 66 citations
Proteomics, 2004; 30 citations
Proteomics, 2004; 108 citations

C2C12 Myogenesis



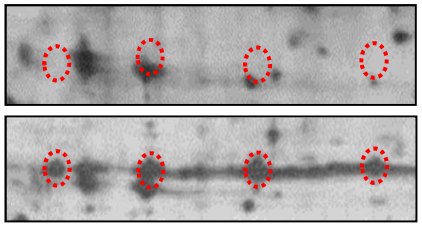
TRIM72 is found in the lipid rafts of C2C12 myotubes.





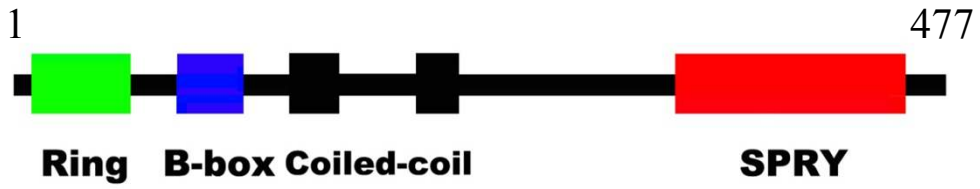
Lee et al., CDD, 2010

TRIM72 gene analysis



Myoblasts

Myotubes

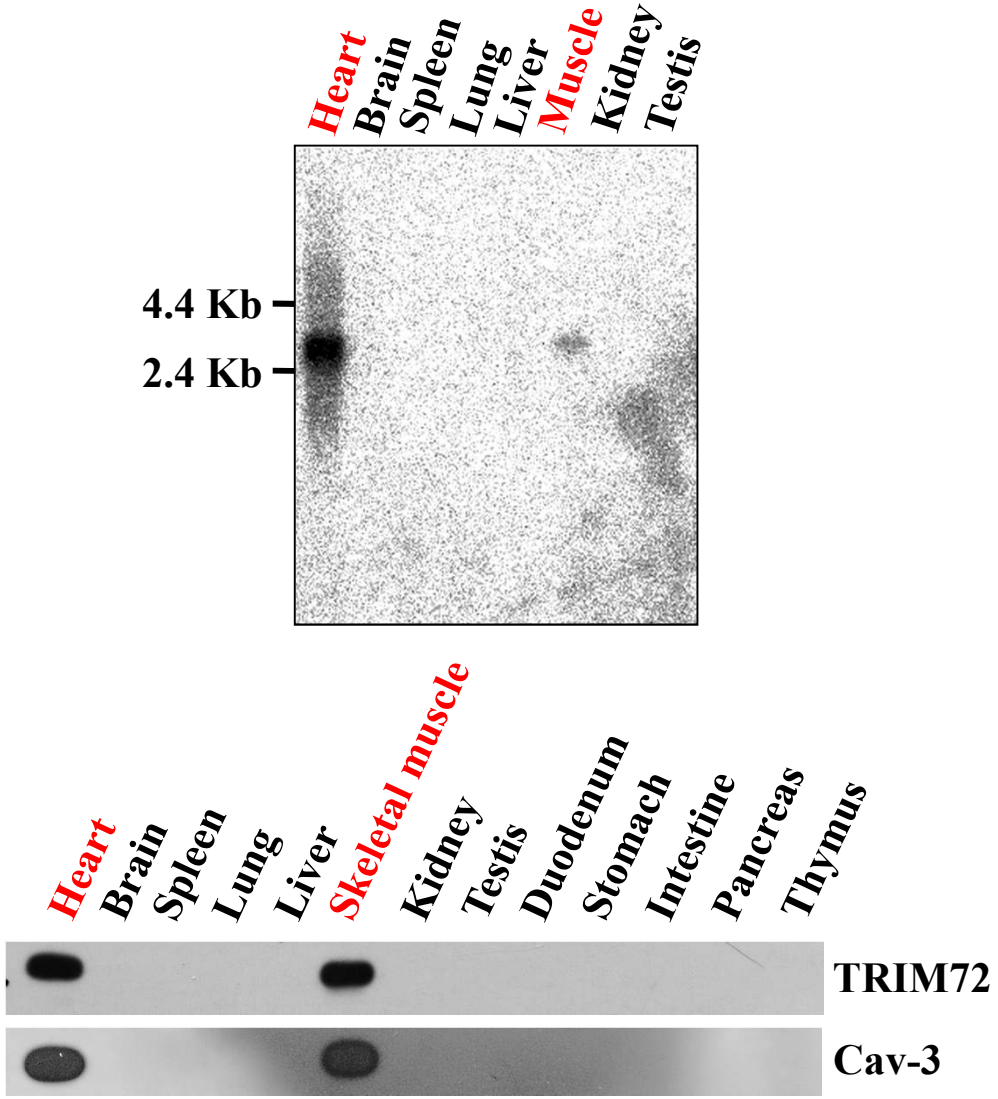


Tripartite Motif (TRIM)
E3 ligase activity

SPla and RYanodine
receptor domain

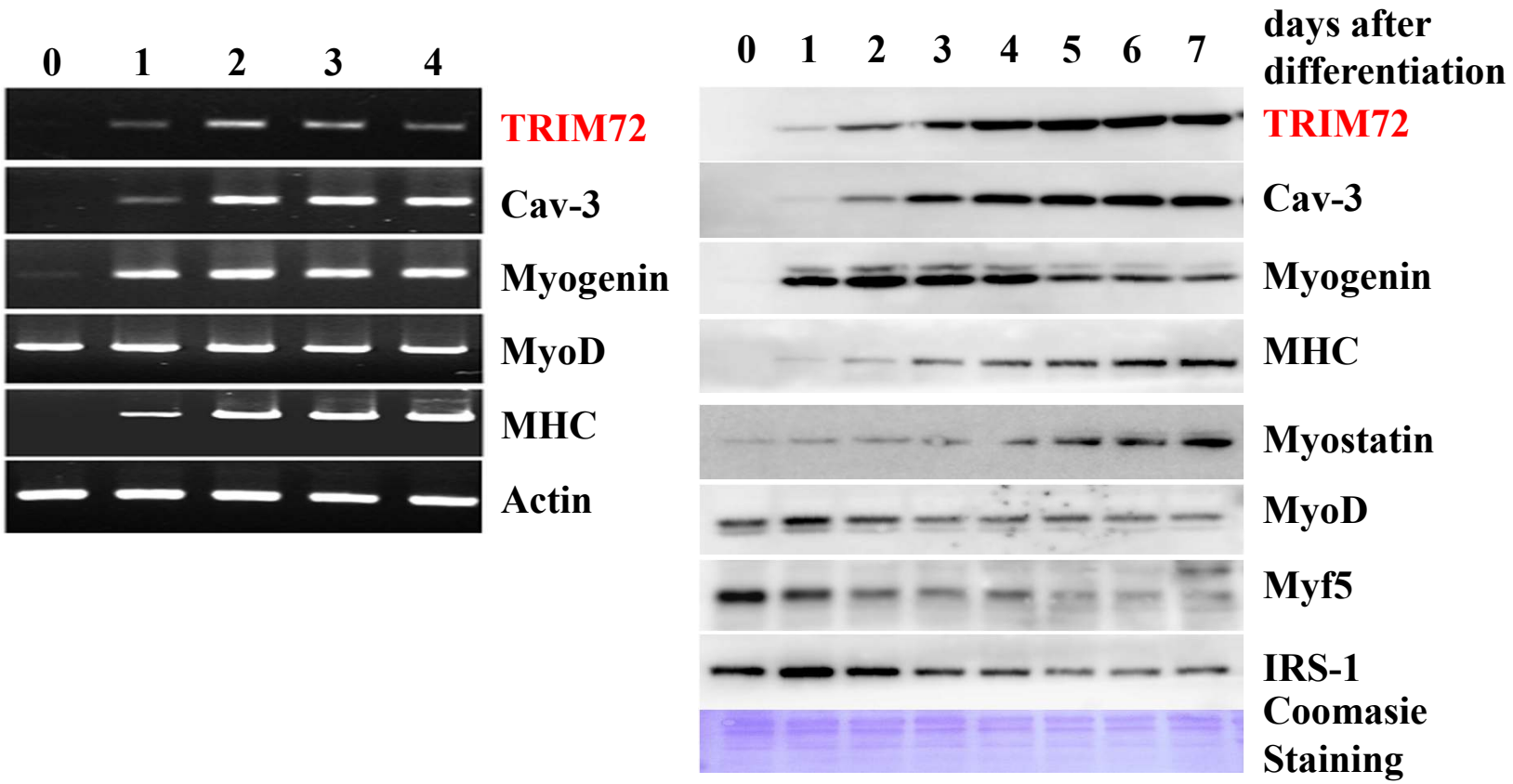
Lee et al., CDD, 2010

TRIM72 is specifically expressed in skeletal and cardiac muscle



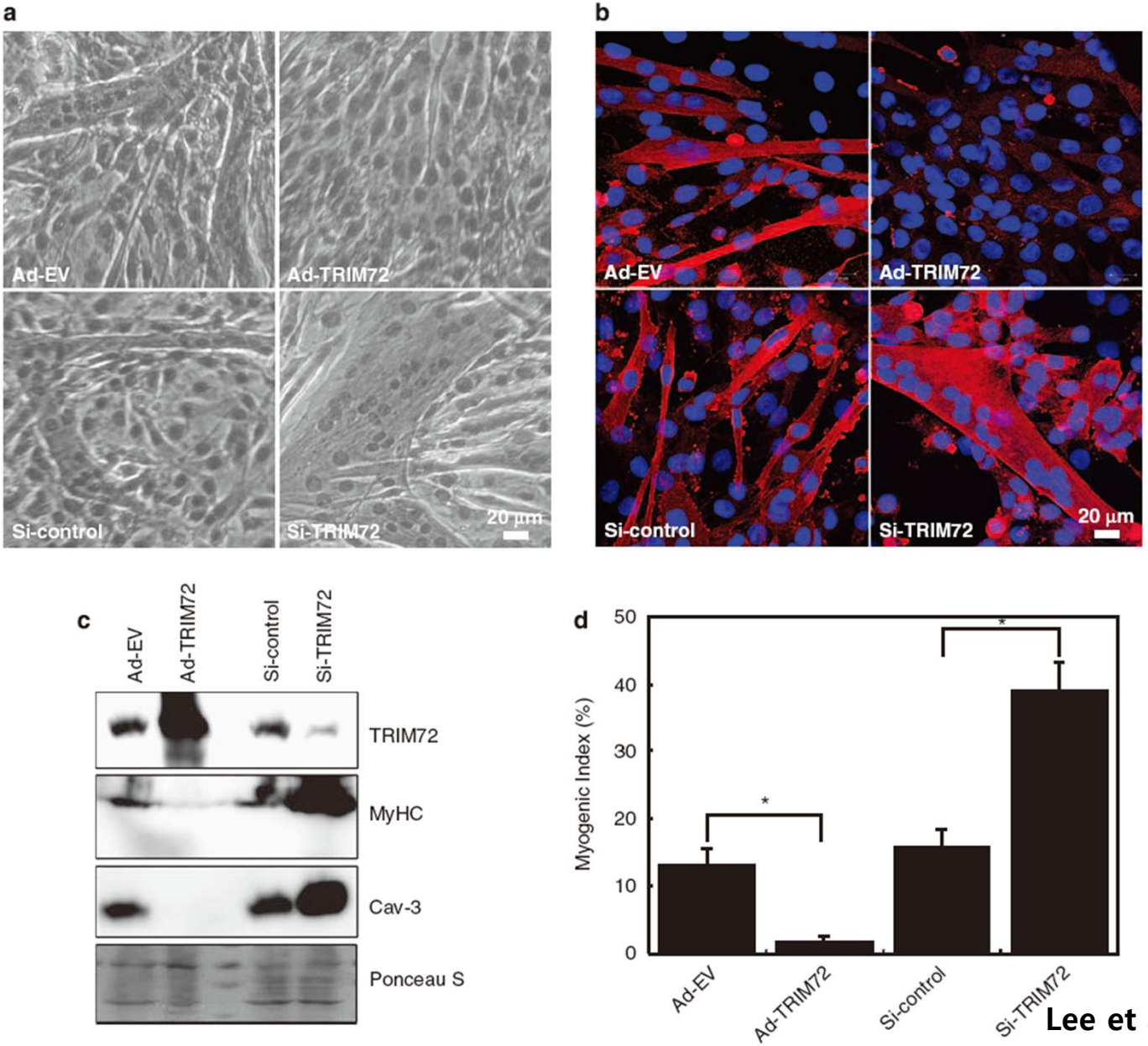
Lee et al., CDD, 2010

TRIM72 is highly expressed during C2C12 myogenesis



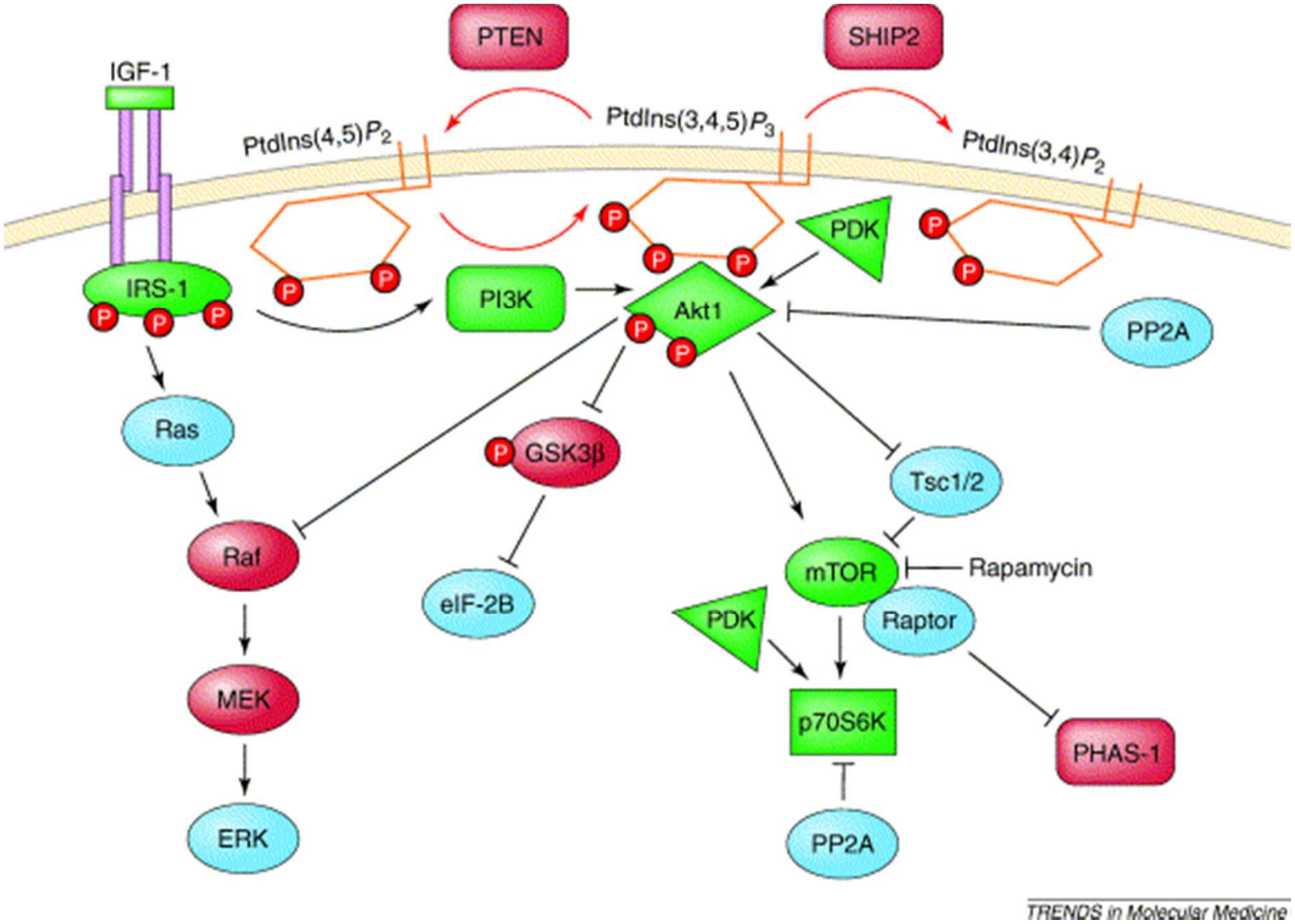
Lee et al., CDD, 2010

TRIM72 is a myogenesis inhibitor

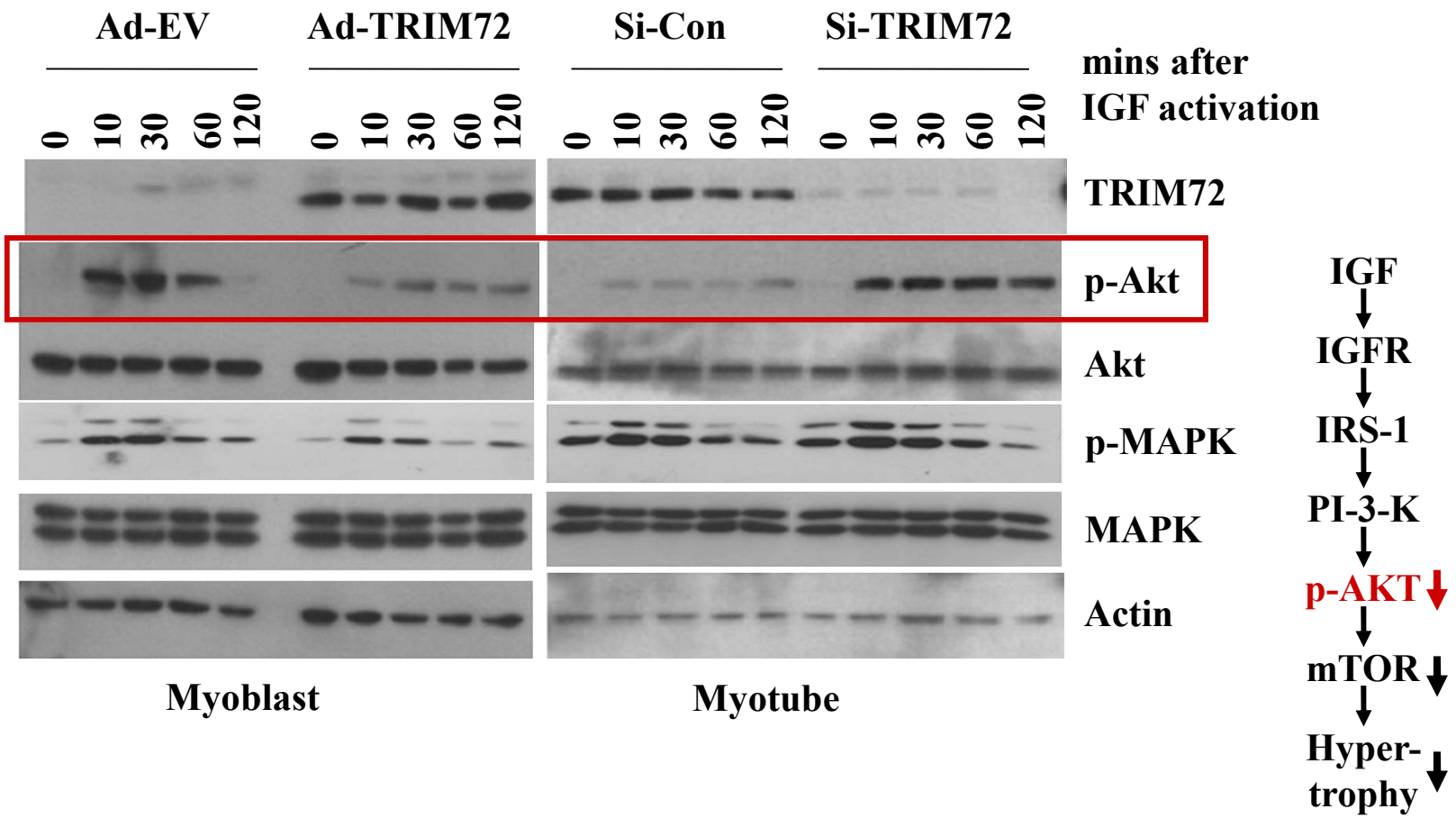


Lee et al., CDD, 2010

What is a molecular target of TRIM72 in IGF signaling?

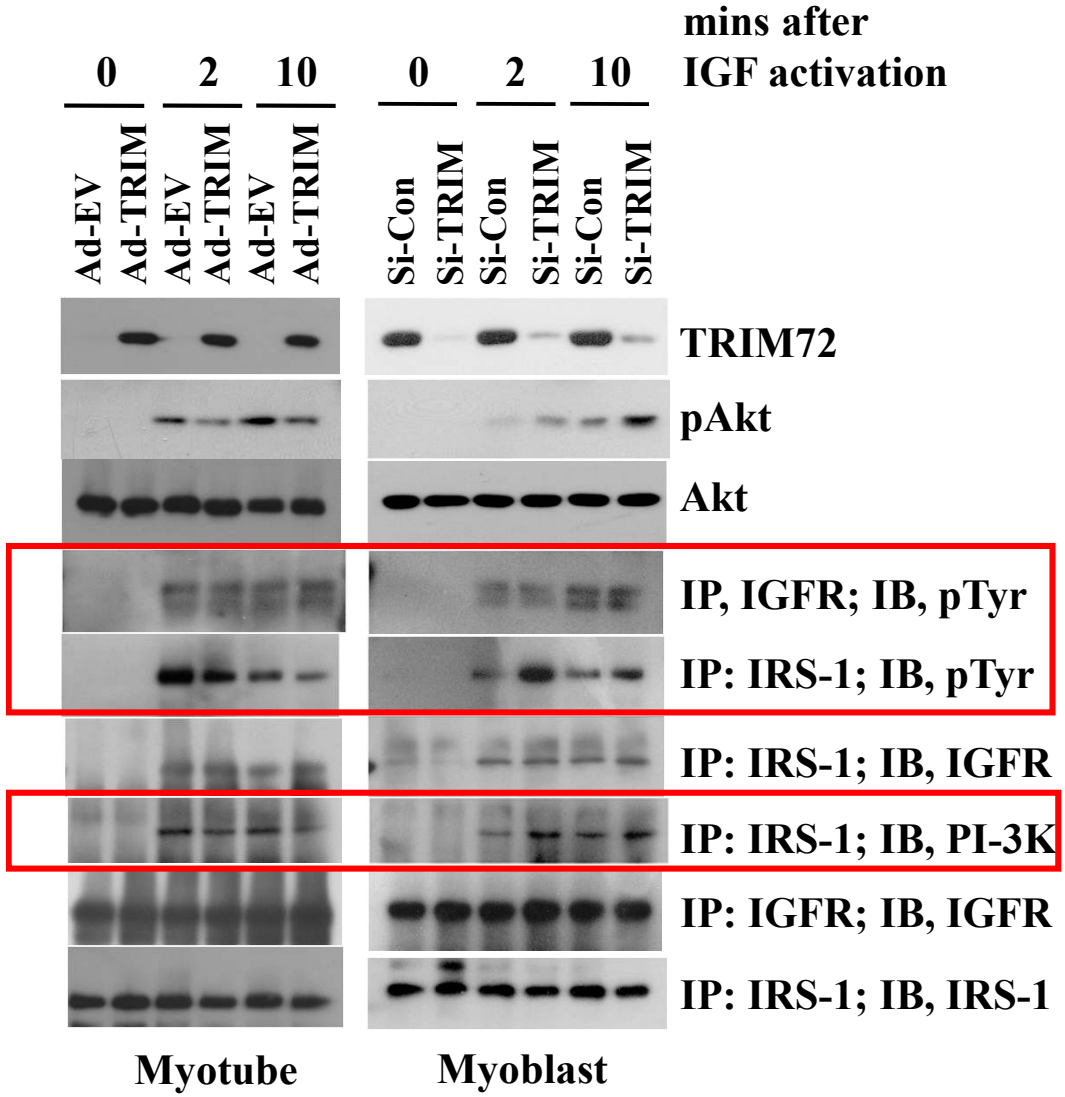


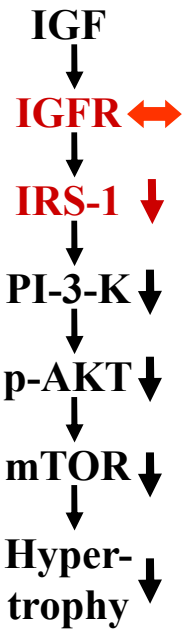
TRIM72 blocks Akt activation in IGF signaling.



Lee et al., CDD, 2010

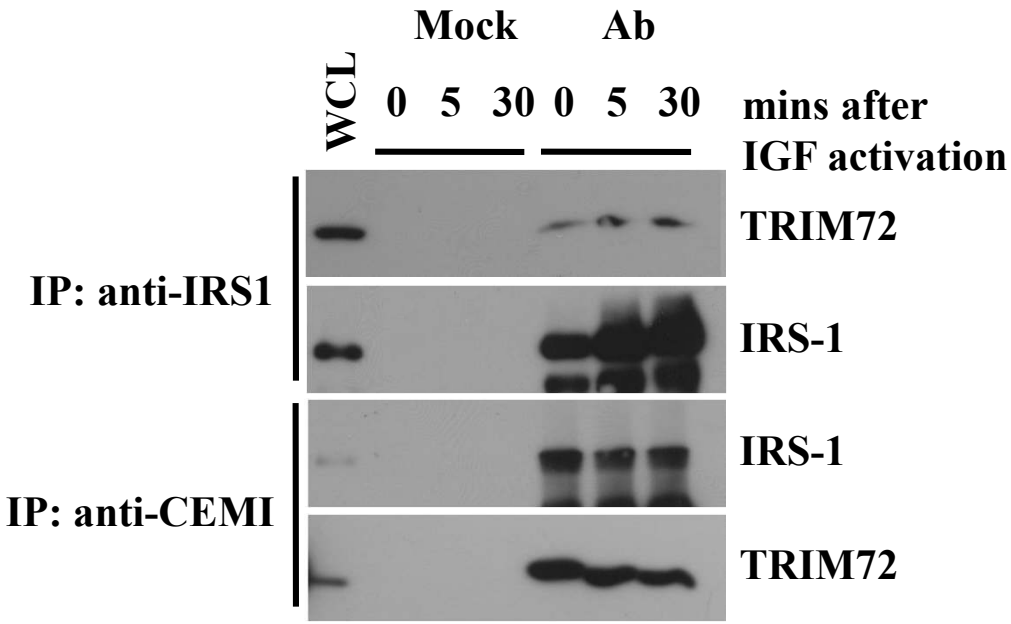
TRIM72 supresses IRS-1 phosphorylation by IGF-1.

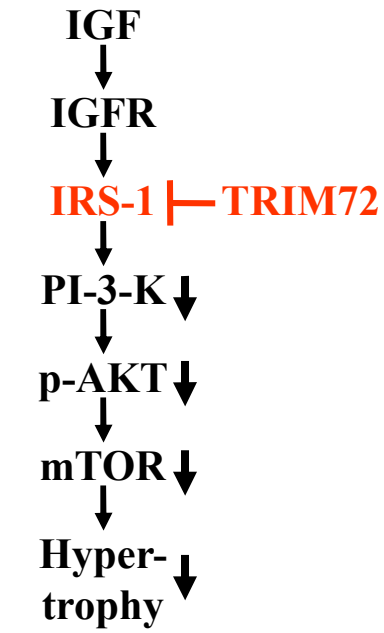




Lee et al., CDD, 2010

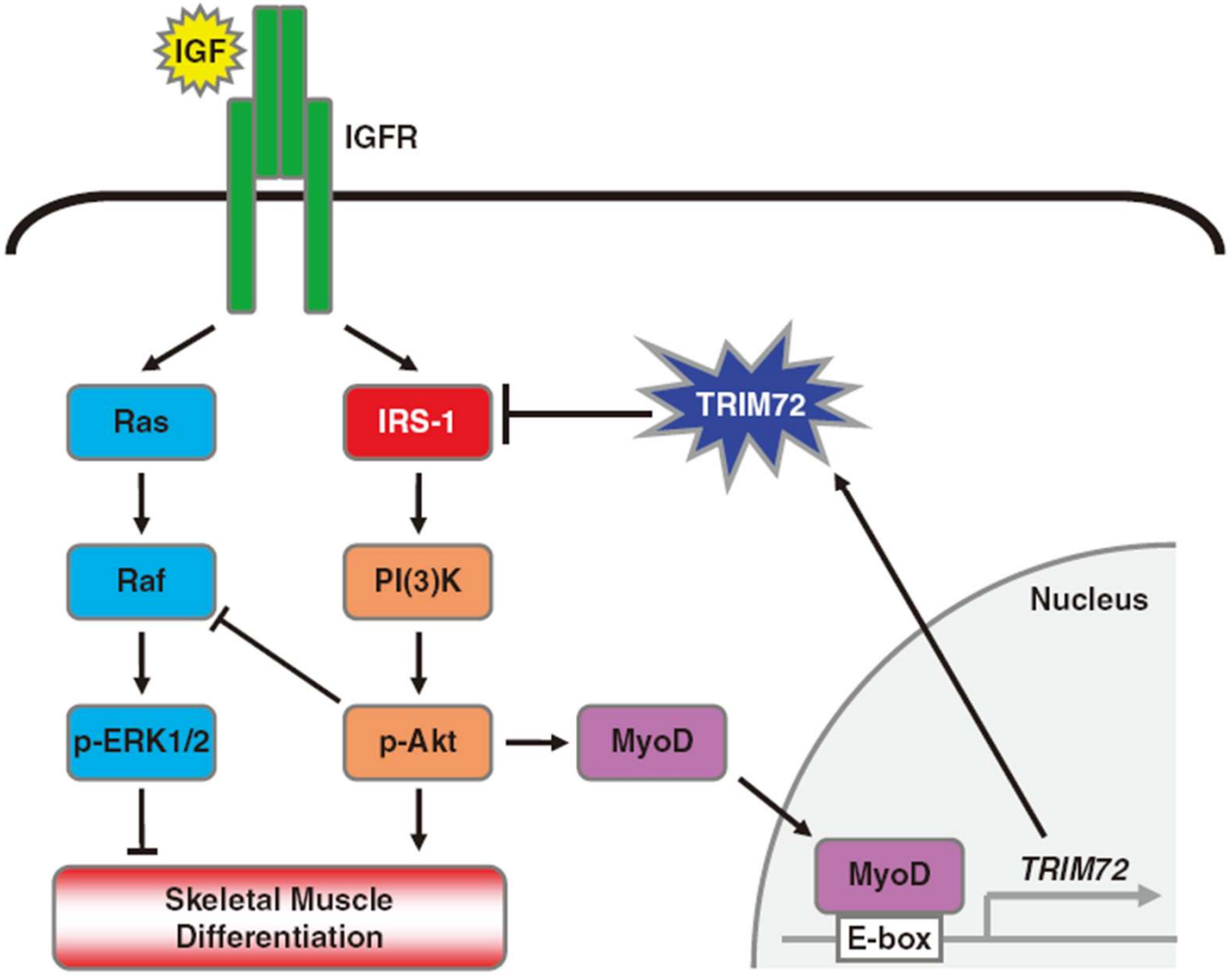
Molecular association of TRIM72 with IRS-1





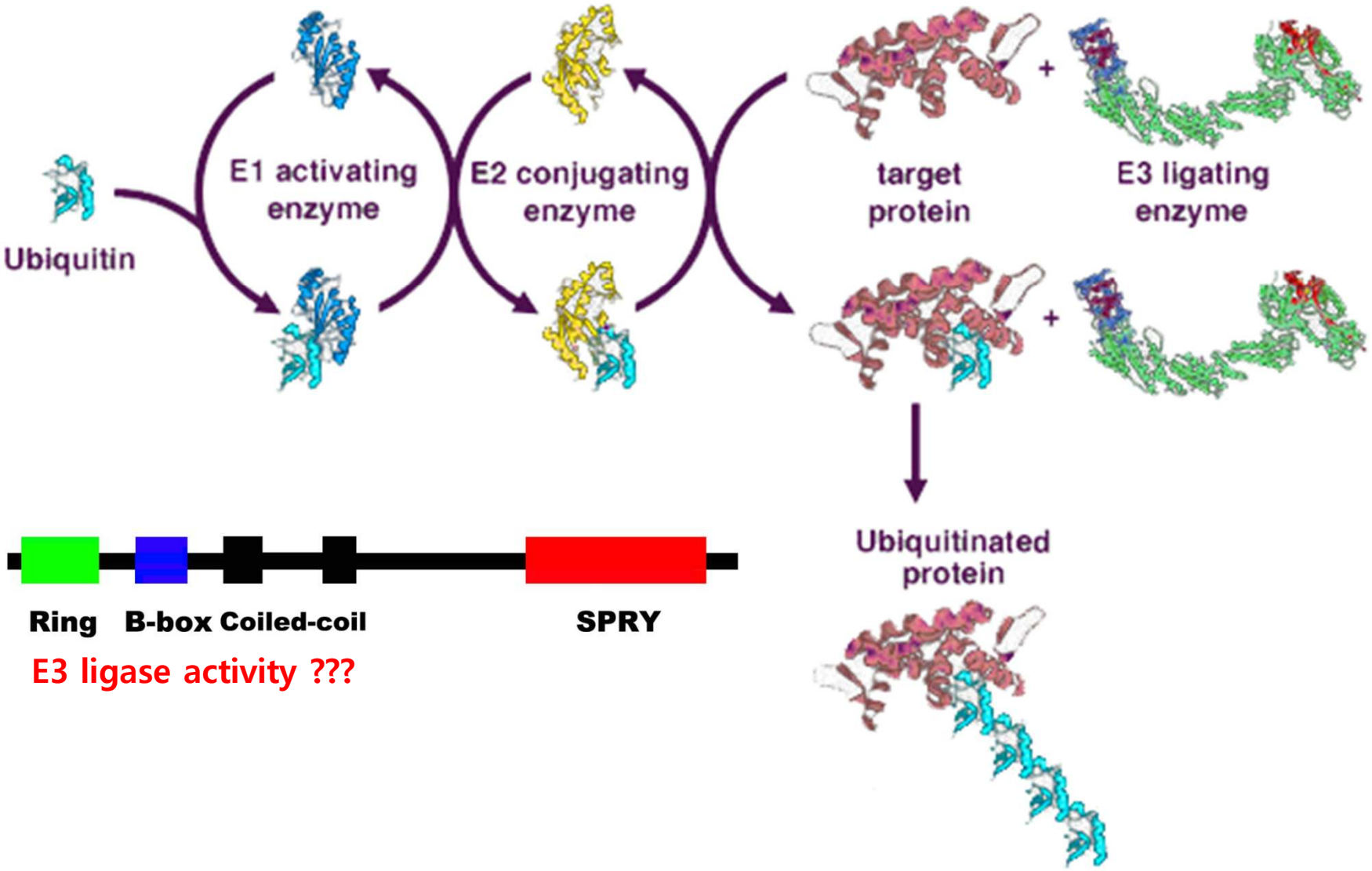
Lee et al., CDD, 2010

TRIM72 negatively regulates myogenesis via targeting IRS-1.



Lee et al., CDD, 2010

Protein Ubiquitination



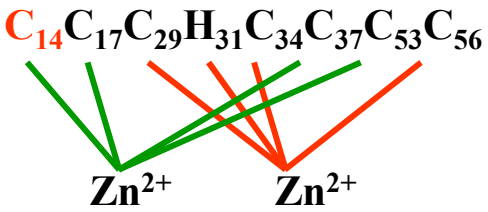
E3 ligase activity ???

Ring Finger domain of TRIM72

Consensus Sequence of Ring Finger Domain, Zn^{2+} binding site
$CX_2CX_{(9-39)}CX_{(1-3)}HX_{(2-3)}C/HX_2CX_{(4-48)}CX_2C$

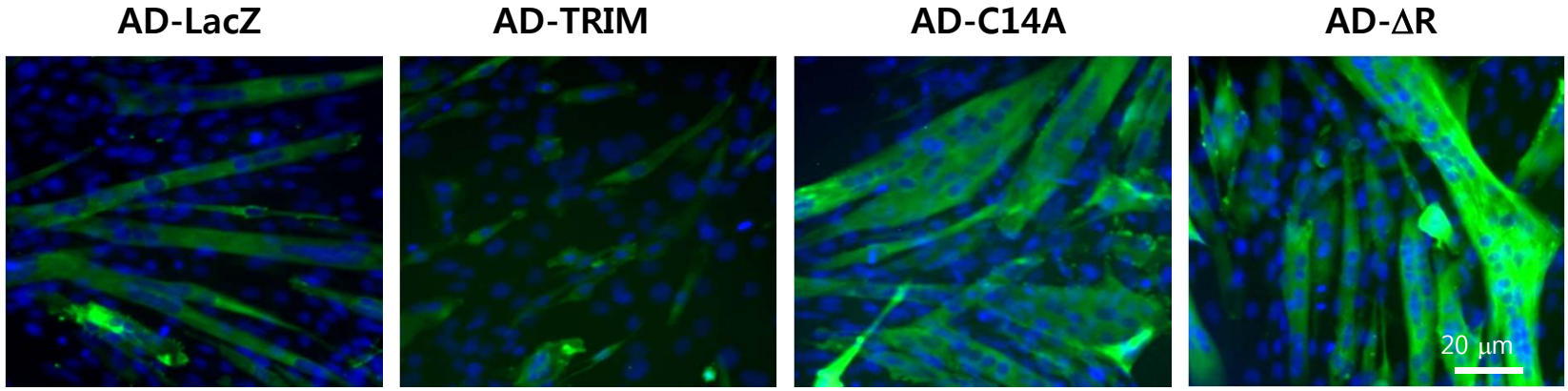
TRIM72 sequence

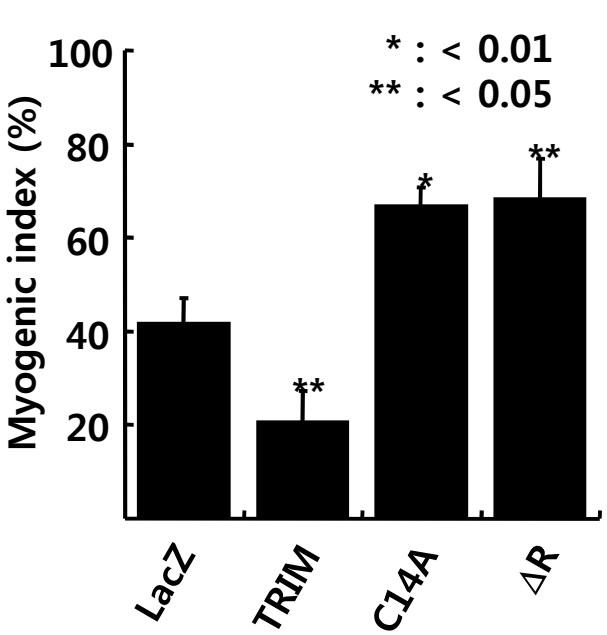
MSAAPGLLHQELSCPLCLQLFDAPVTAECGHSFCRACLGRVAGEPAADGTVLC
PCCQAPTRPQALSTNLQLARLVEGLAQVPQGHCEEHLDPLSIYCEQDRALVCGV
CASLGSHRGHRLLPAAEAHARLKTQLPQQKLQLQEACMRKEKSVAVLEHQLVV
EETVRQFRGAVGEQLGKMRVFLAALEGSLDCEAERVRGEAGVALRRELGSLNS
YLEQLRQMEKVLEEVADKPQTEFLMKYCLVTSRLQKILAESPPPARLDIQLPIISD
DFKFQVWRKMFRALMPALEELTFDPSSAHPSLVVSSSGRRVECSEQKAPPAGED
PRQFDKAVAVVAHQQLSEGEHYWEVDVGDKPRWALGVIAAEAPRRGRLHAVPS
QGLWLLGLREGKILEAHVEAKEPRALRSPERRPTRIGLYLSFGDGVLSFYDASD
ADALVPLFAFHERLPRPVYPFFDVCWHDKGKNAQPLLLVGPEGAEA

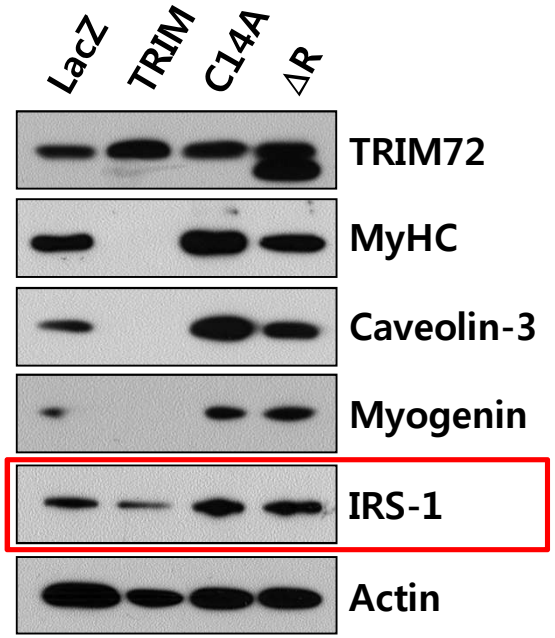


TRIM72 C14A mutant ?
TRIM72 ΔRING mutant ?

RING domain of TRIM72 is essential for the negative regulation of myogenesis.

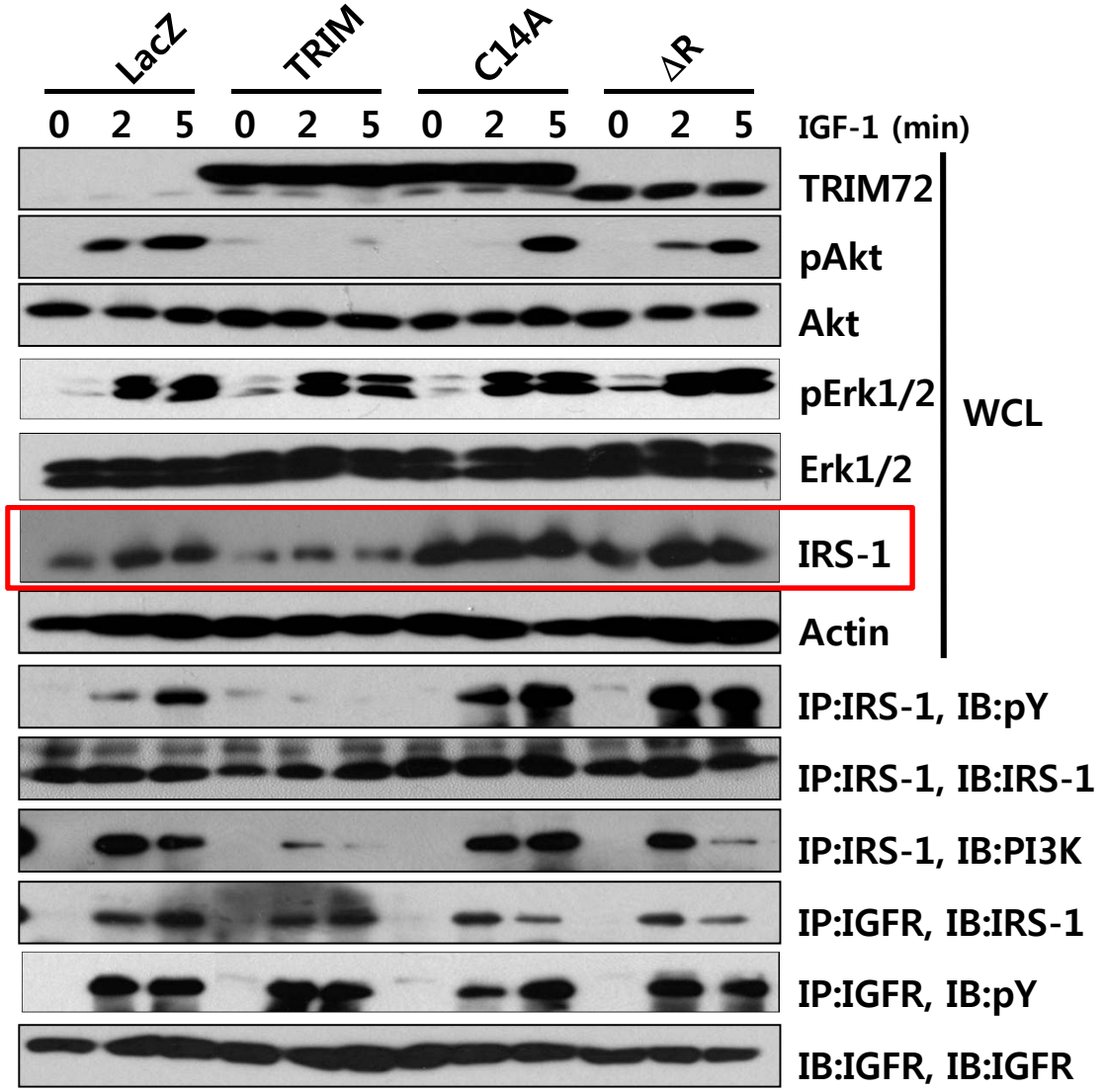


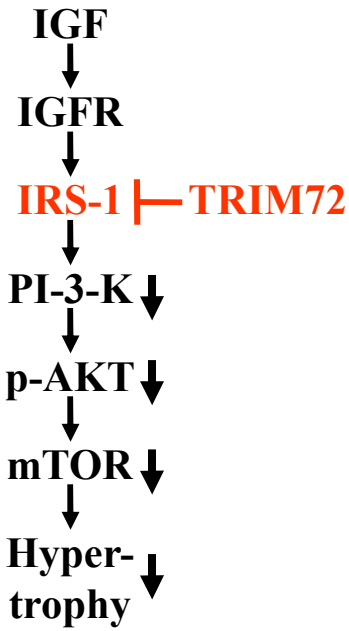




C2C12 myotubes

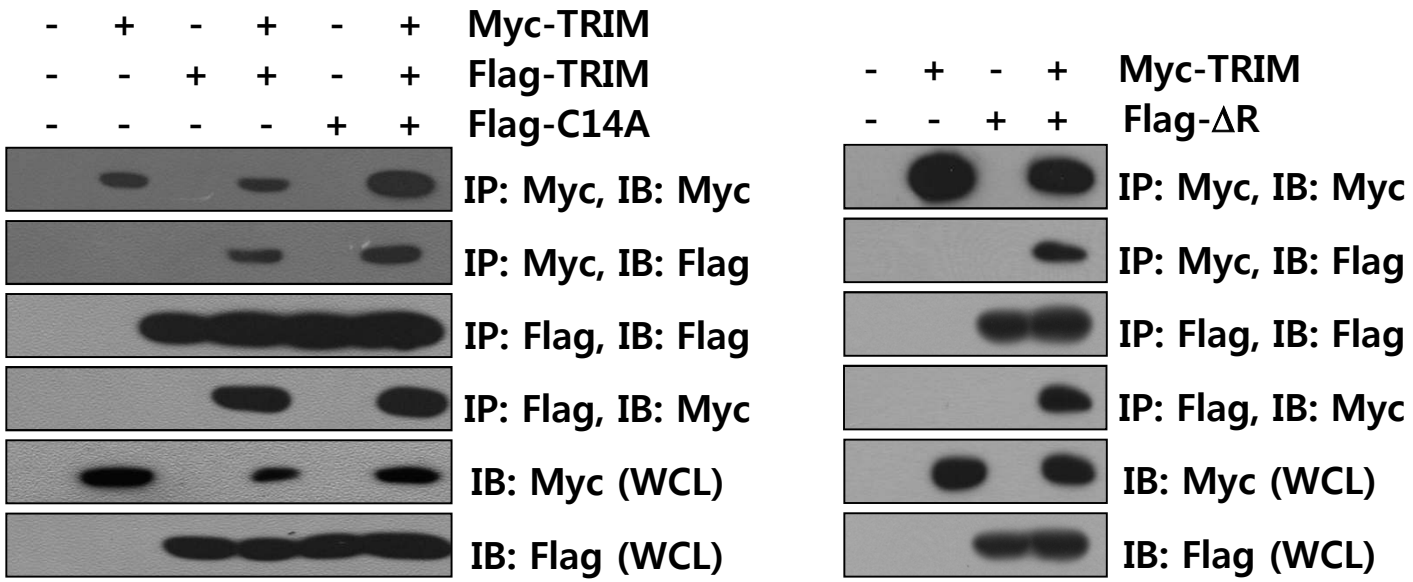
RING domain of TRIM72 is required for the negative regulation of IGF signaling.





C2C12 myoblasts

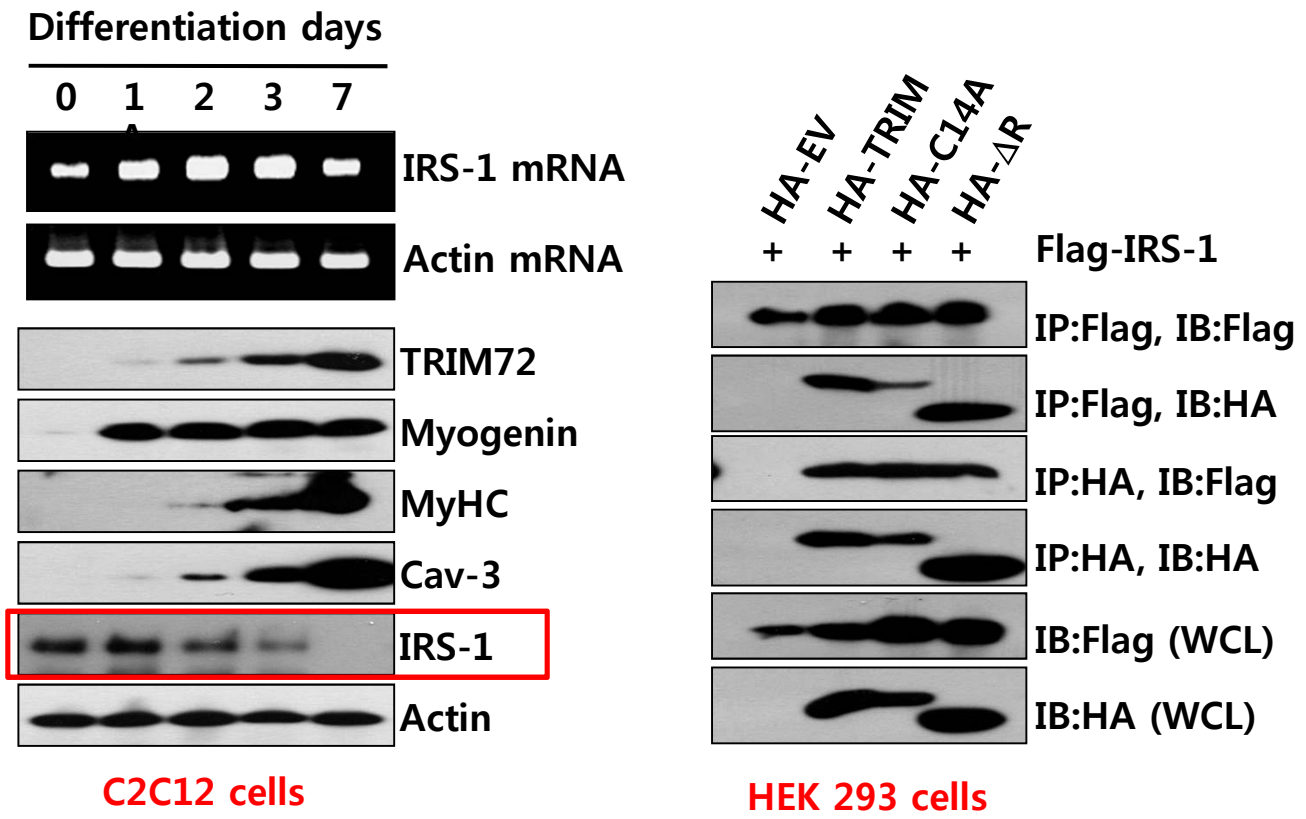
RING domain-lacking TRIM72 mutants work as dominant negative forms of endogenous TRIM72.



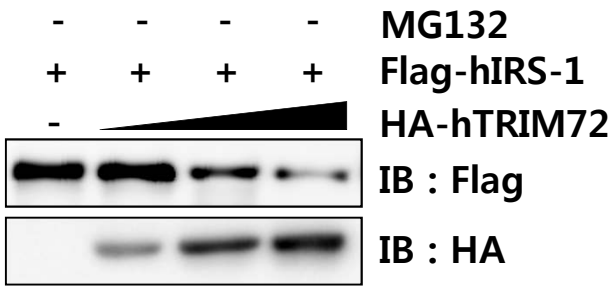
TRIM72 oligomerization

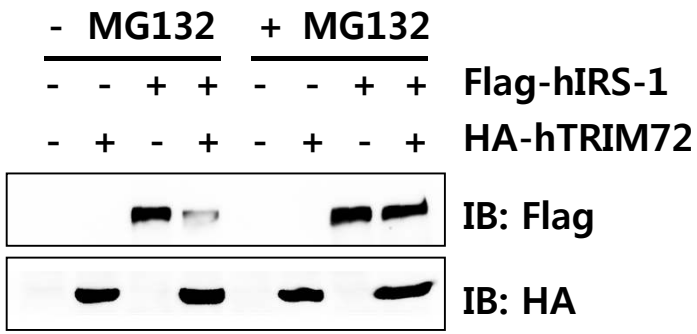
HEK 293 cells

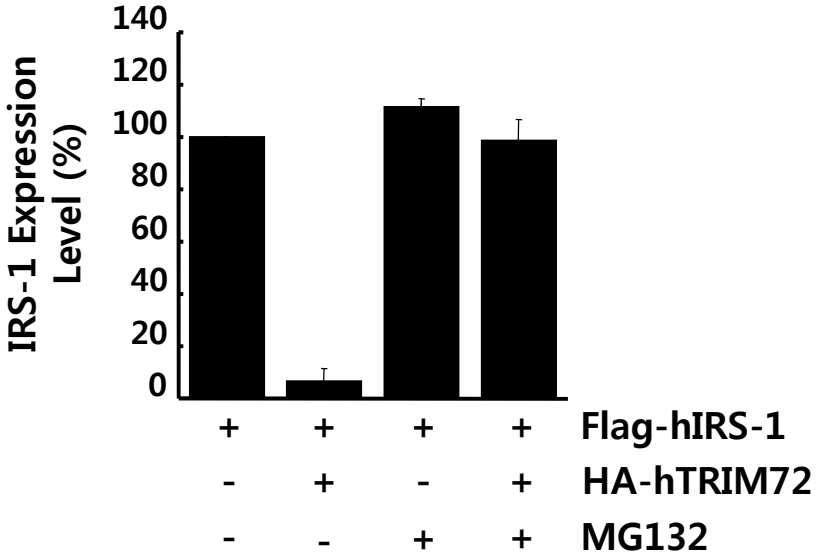
RING domain does not prevent binding of TRIM72 to IRS-1



RING domain of TRIM72 is required for IRS-1 degradation.

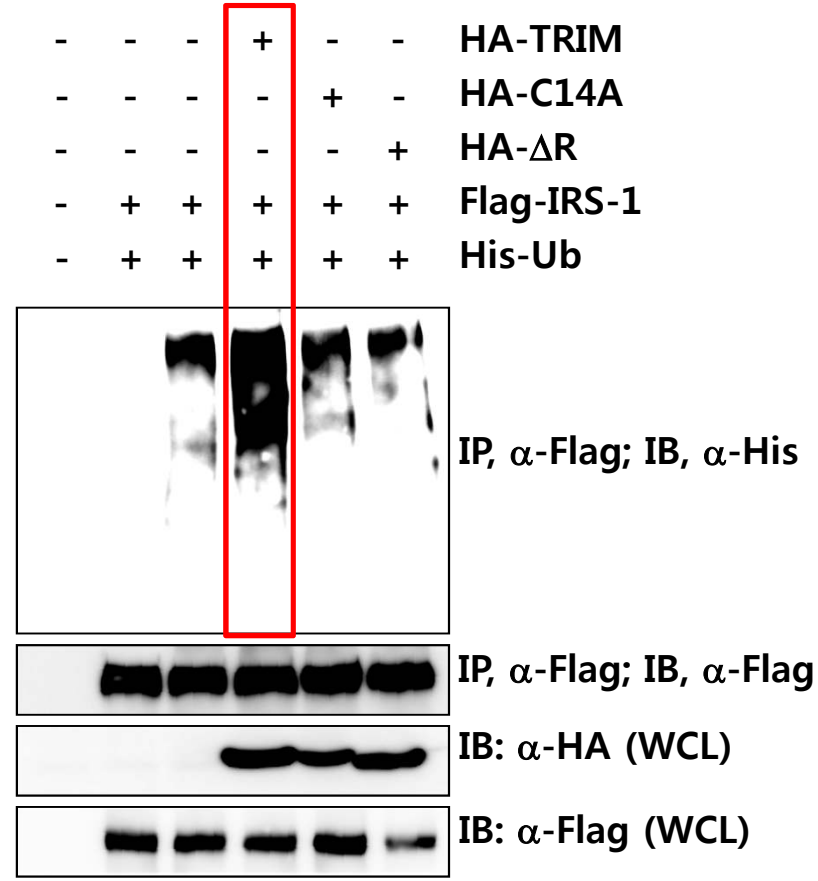






HEK 293 cells

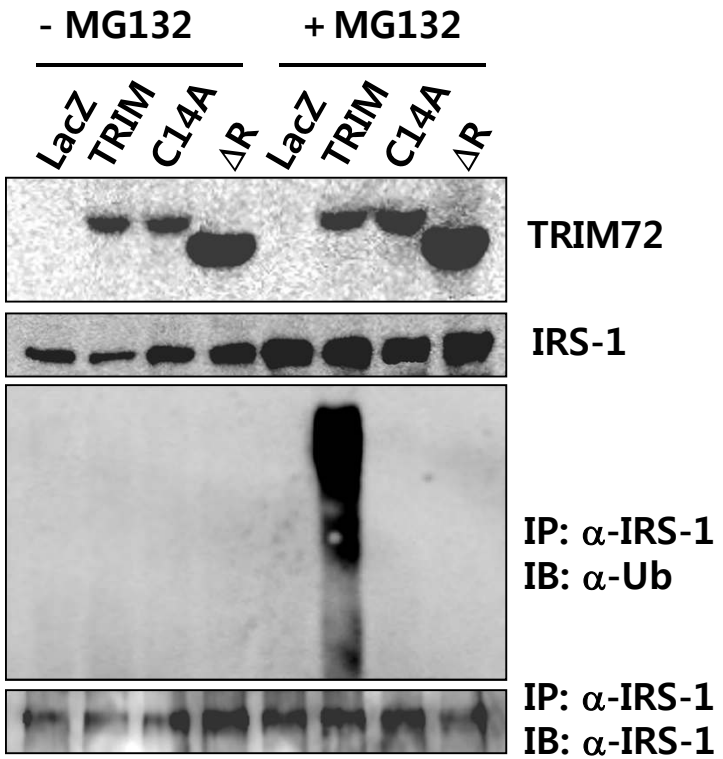
RING domain of TRIM72 is required for IRS-1 ubiquitination.



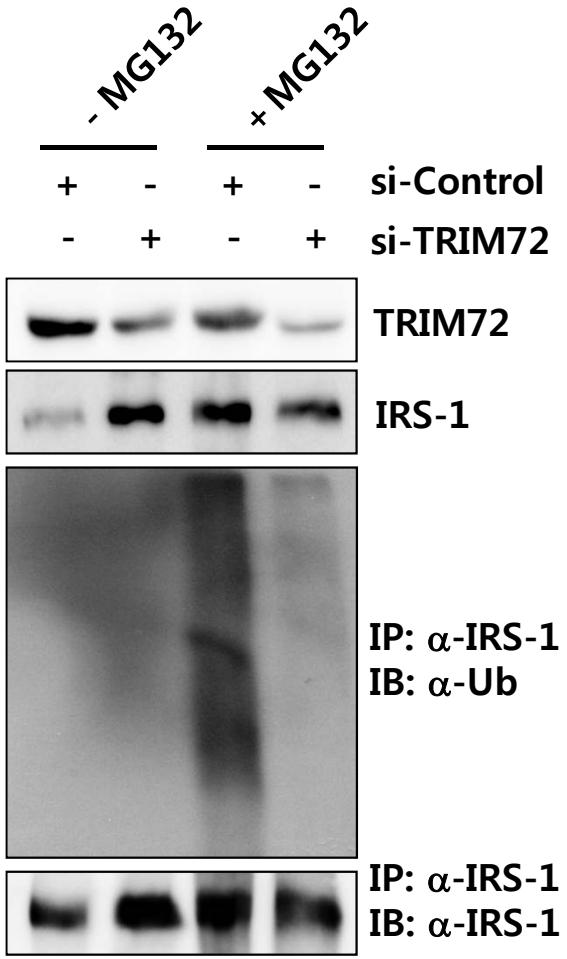
In the presence of MG132

HEK 293 cells

RING domain of TRIM72 is required for IRS-1 ubiquitination.

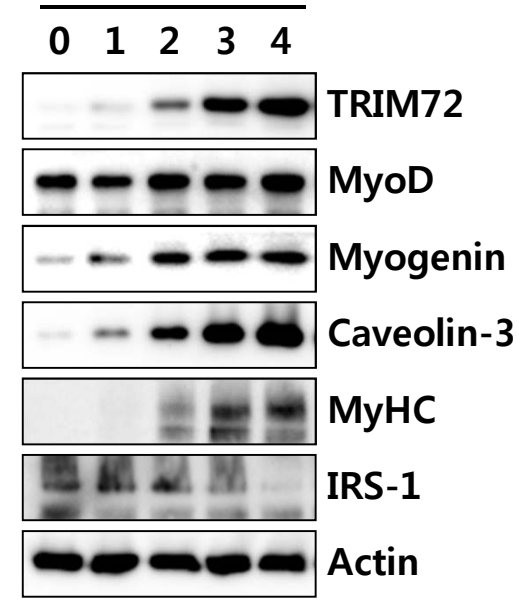


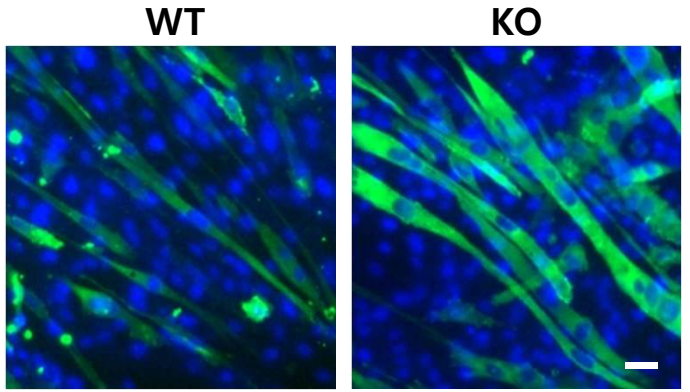
C2C12 Myoblasts

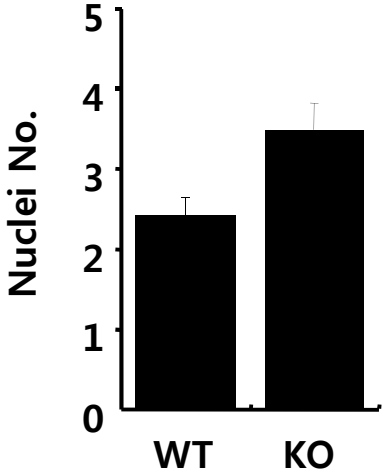


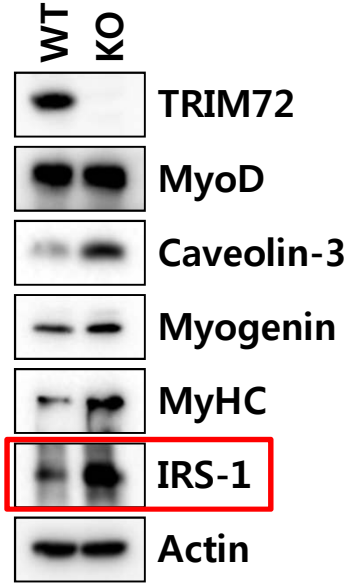
C2C12 Myotubes

TRIM72 disruption enhances MyoD-driven myogenesis in MEFs.



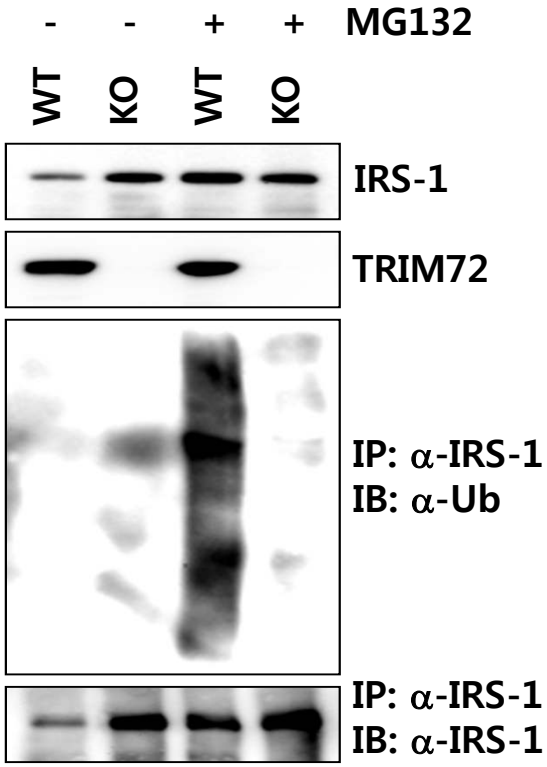






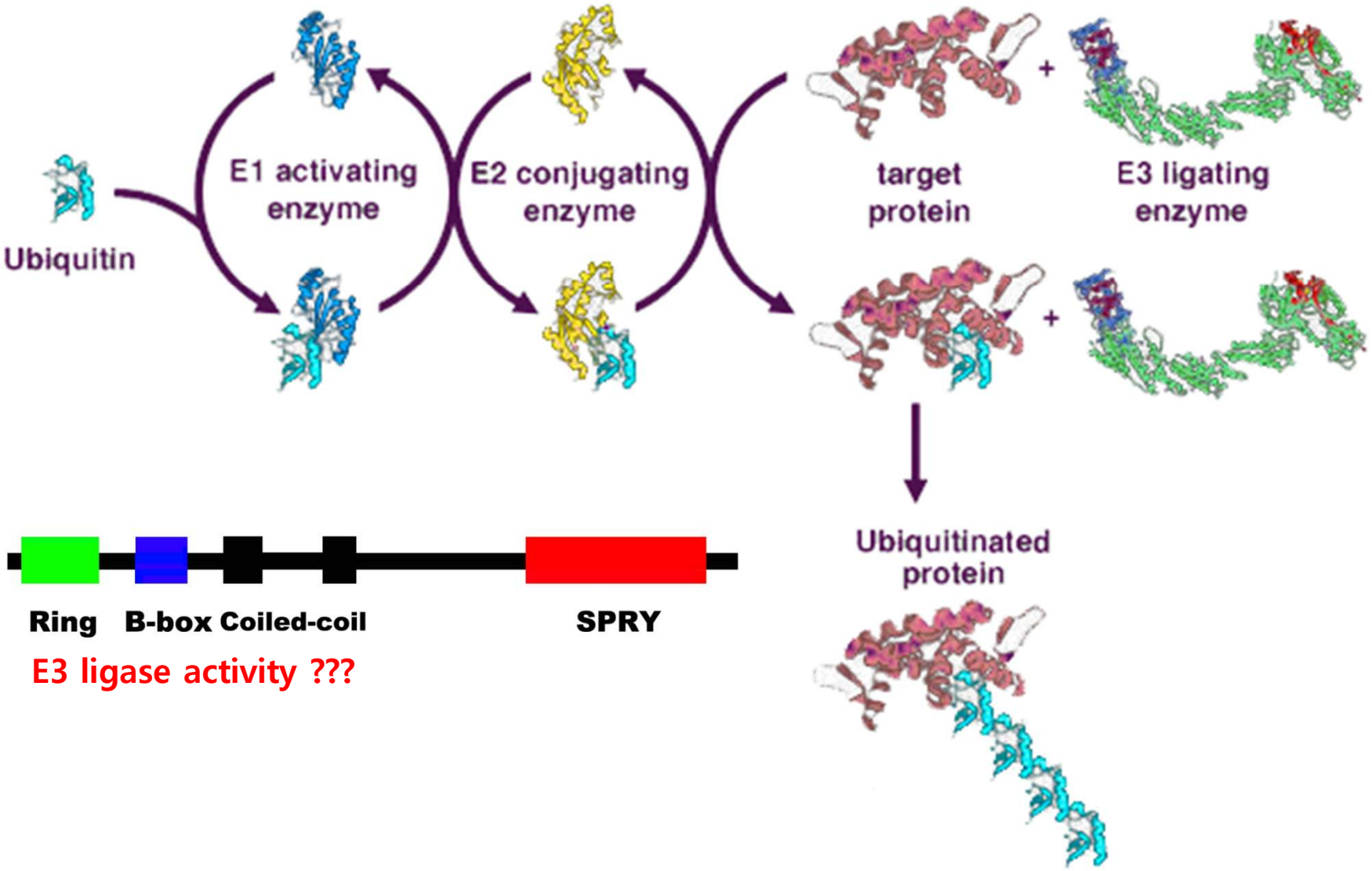
MyoD-driven myotubes from TRIM72$^{+/+}$ and $^{-/-}$ MEFs

TRIM72 disruption abolishes IRS-1 ubiquitination in MyoD-driven myotubes of MEFs.



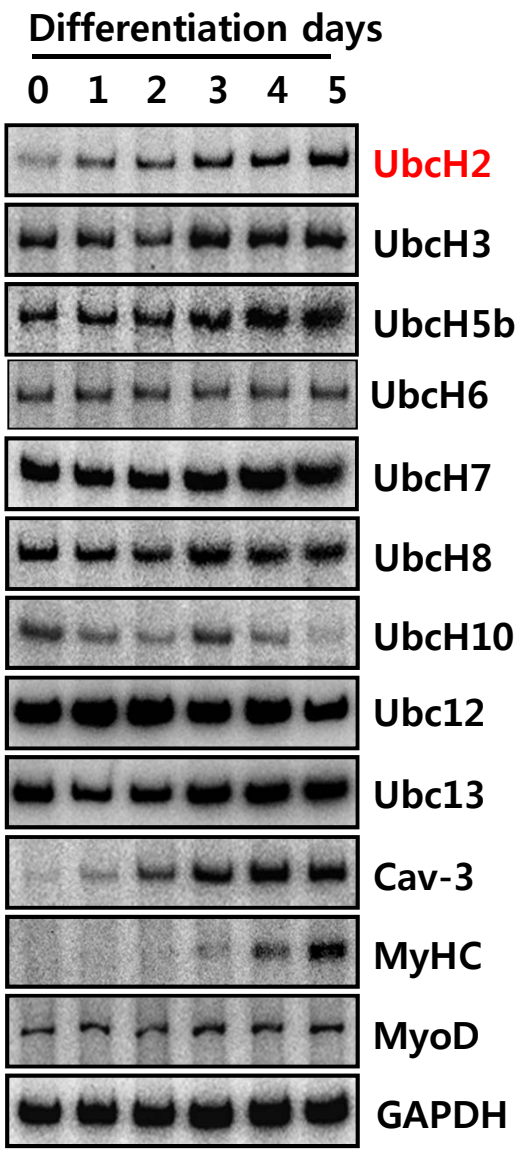
MyoD-driven myotubes from TRIM72$^{+/+}$ and $^{-/-}$ MEFs

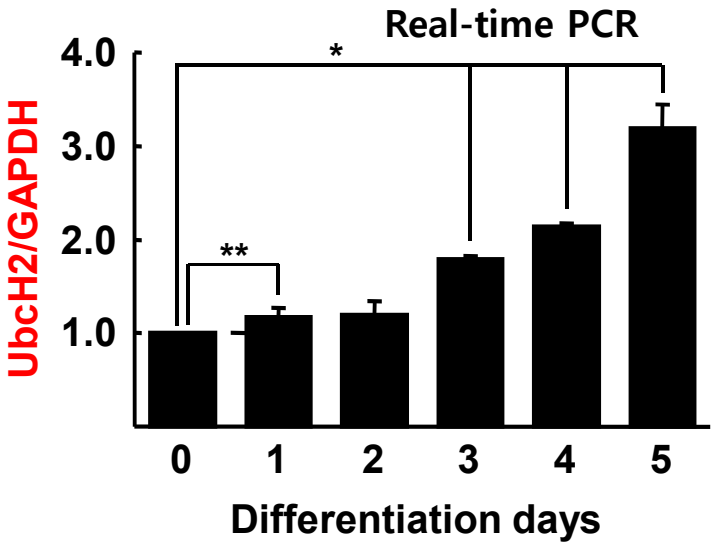
Protein Ubiquitination

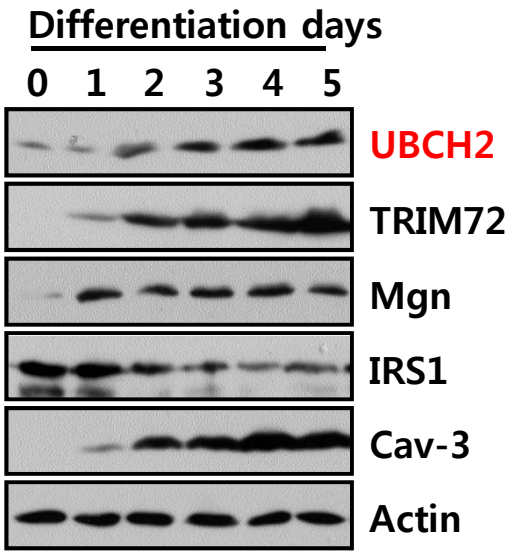


E3 ligase activity ???

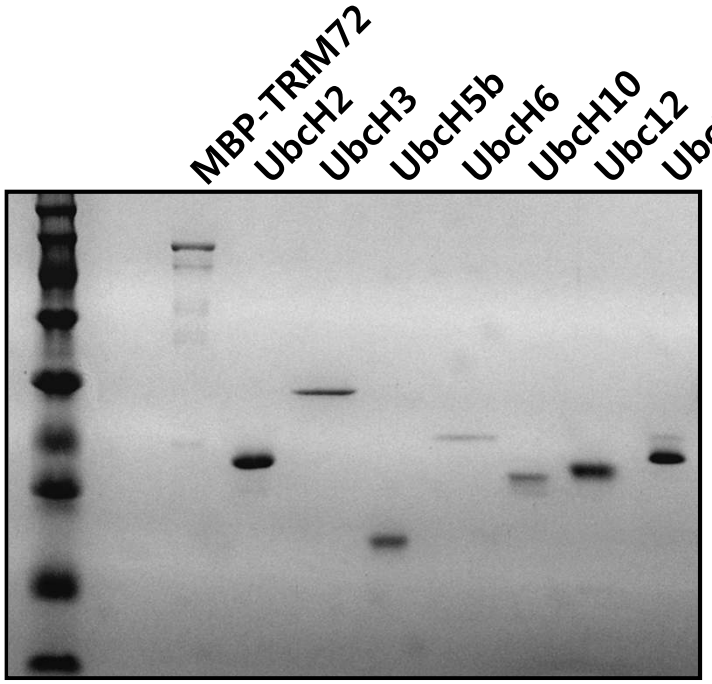
UbcH2 mRNA and protein are increased during C2C12 myogenesis



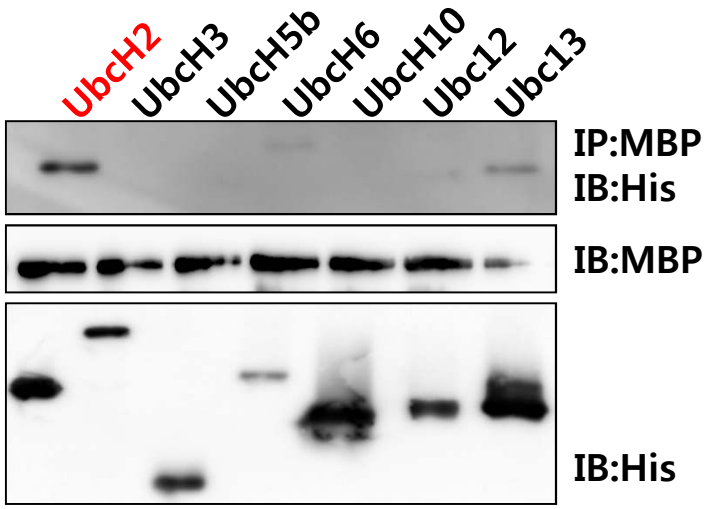




Molecular association of TRIM72 with UbcH2

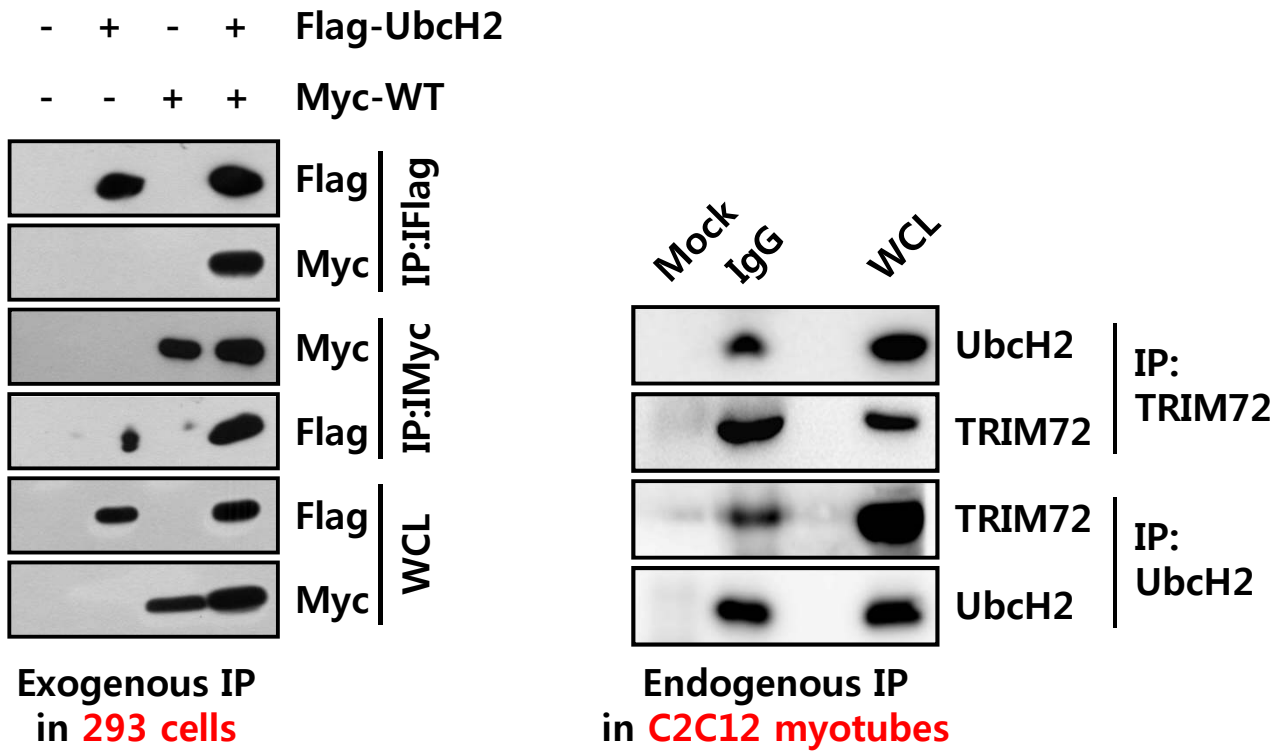


Purified TRIM72 and E2 enzymes

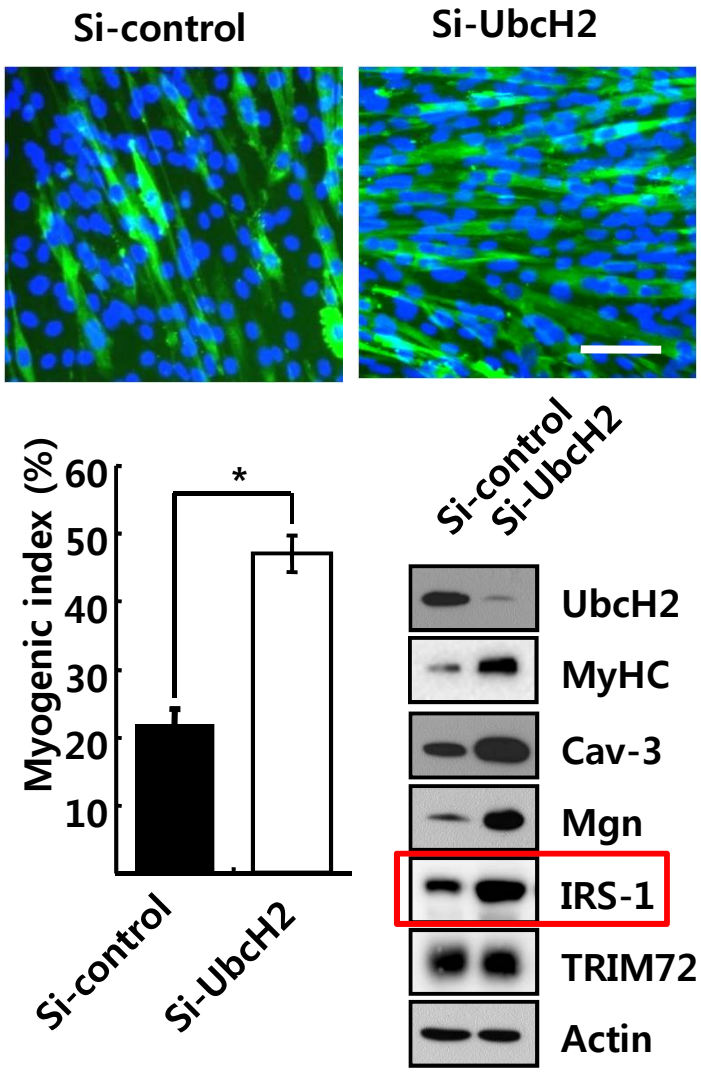


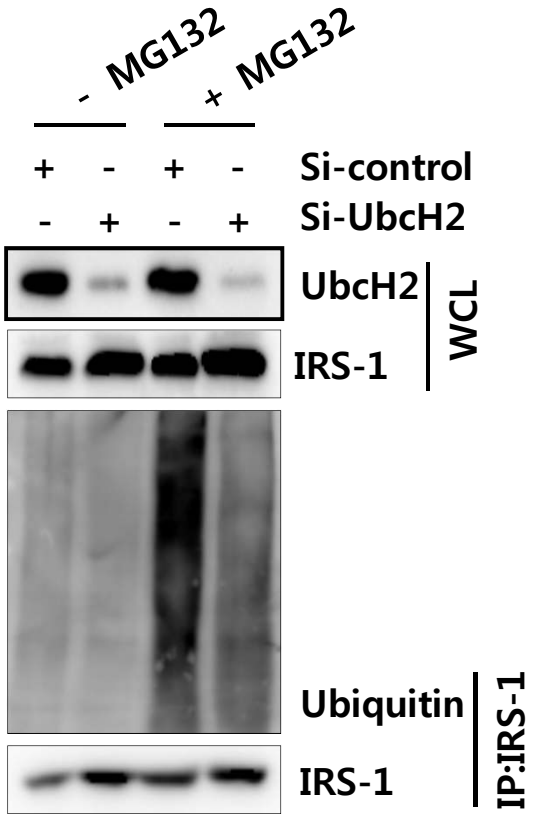
In vitro binding assay

Molecular association of TRIM72 with UbcH2



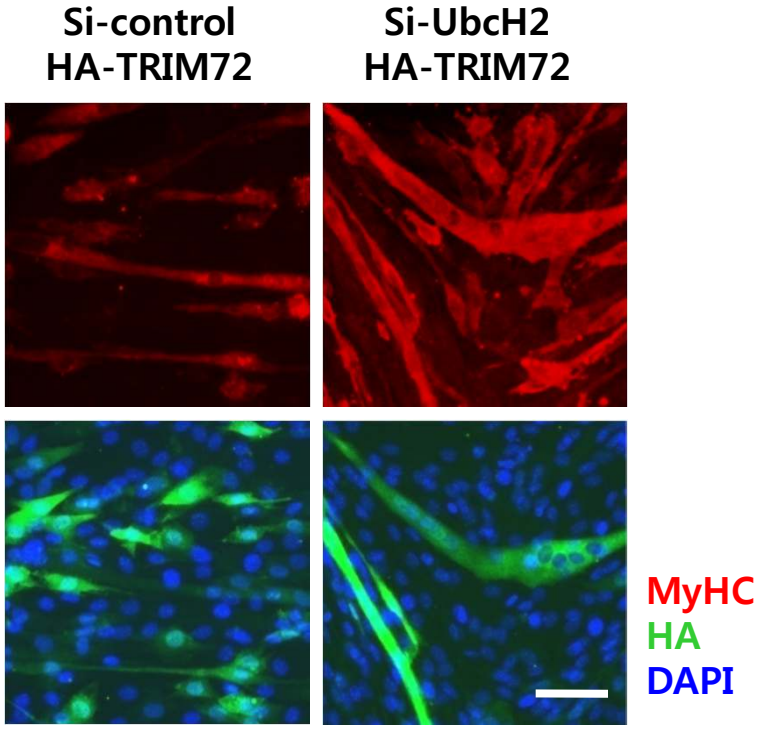
UbcH2 knockdown enhances myogenesis by inhibiting IRS-1 ubiquitination.

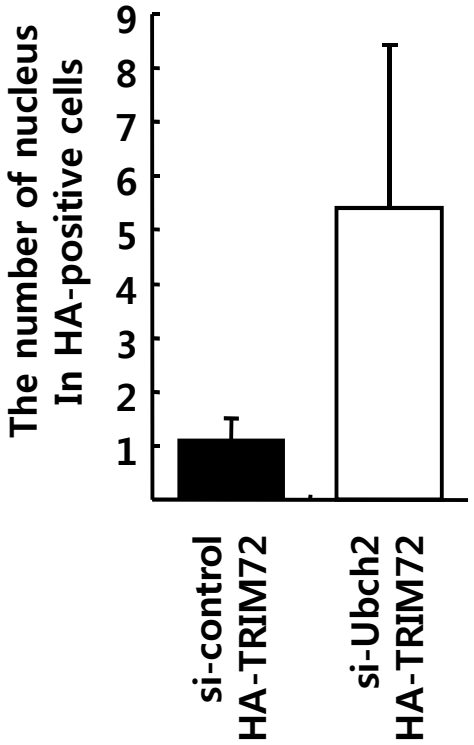




C2C12 myotubes

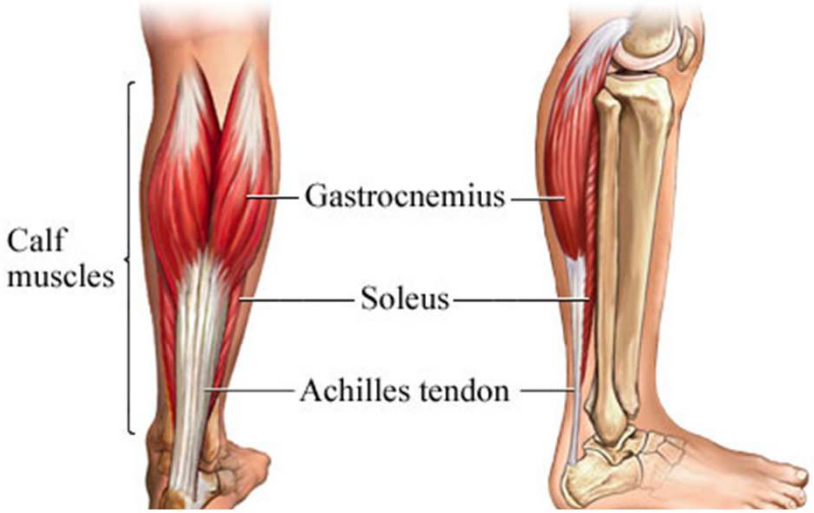
TRIM72 inhibition of myogenesis is released by UbcH2 knockdown



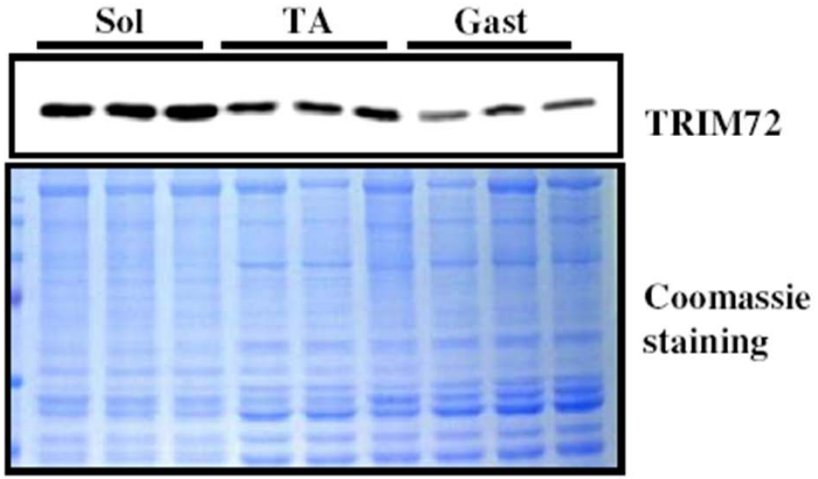


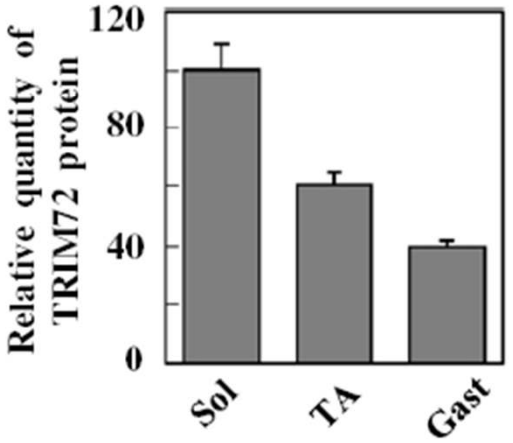
C2C12 myotubes

TRIM72 is highly expressed in soleus muscle.



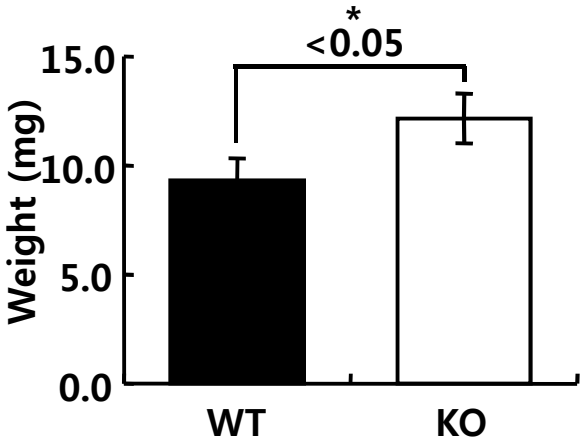
	soleus	gastrocnemius
Color	Red muscle	White muscle
Size	Smaller	Larger
Fatigue	Resistant	Sensitive
TRIM72	High	Low

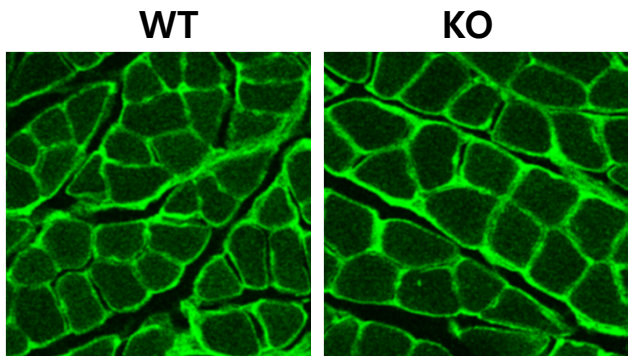


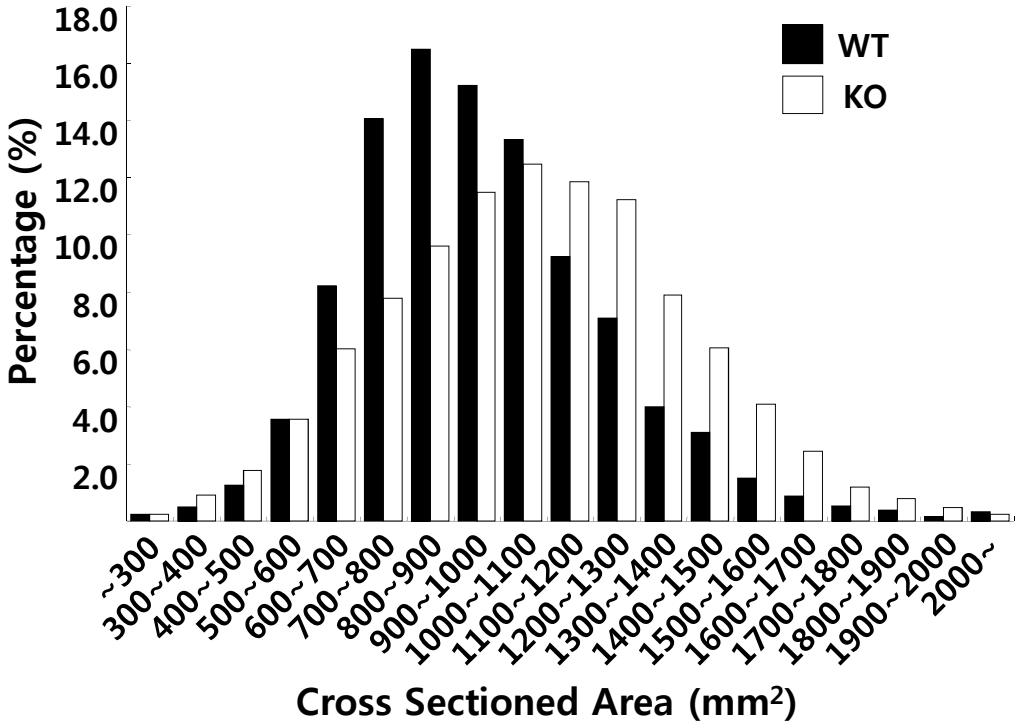


Jung and Ko, BBRC, 2010

Skeletal muscle size is increased in TRIM72-disrupted mice.

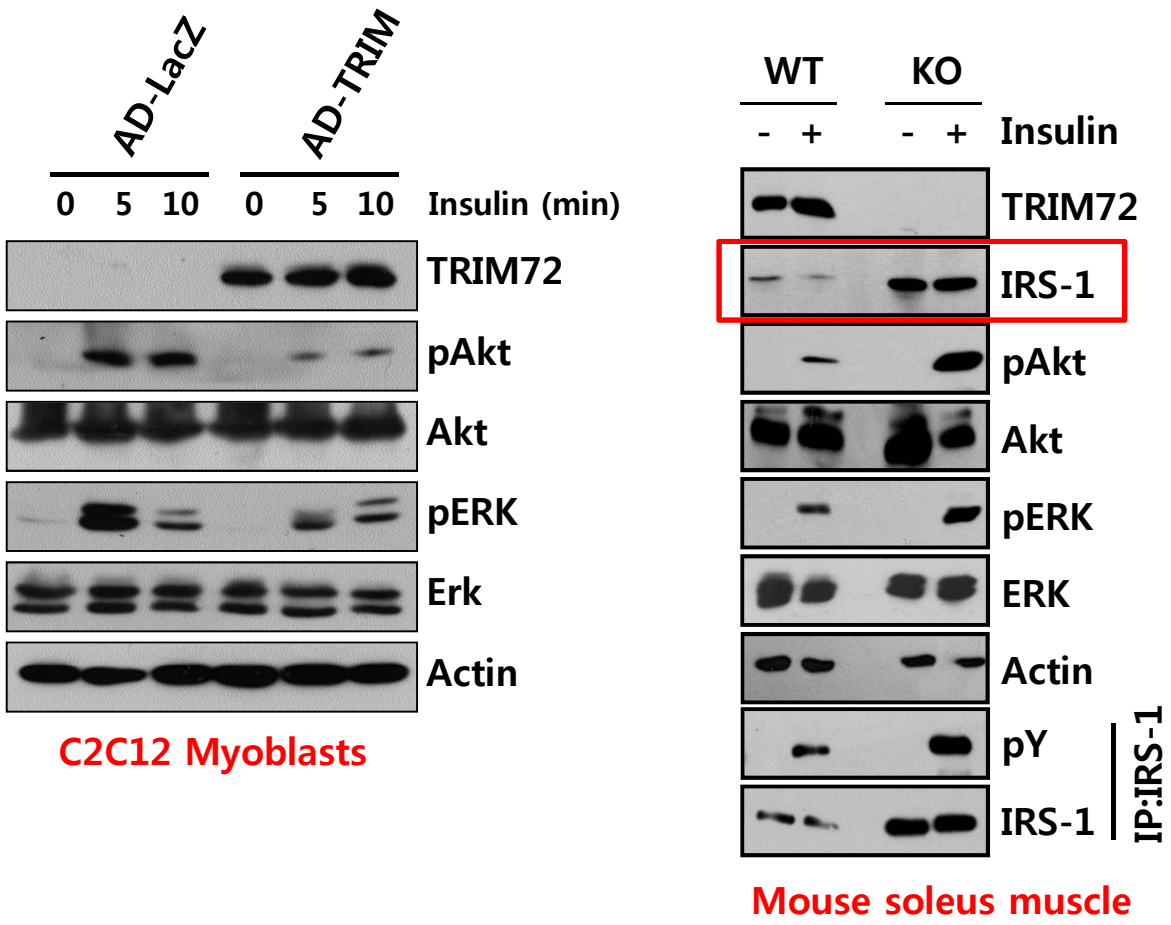




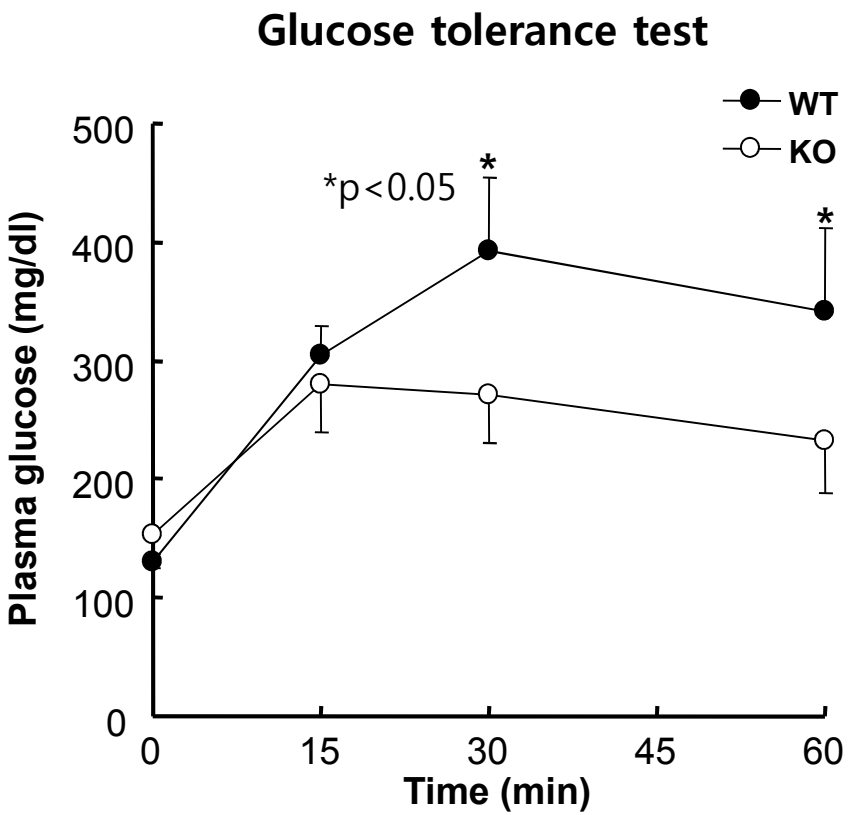


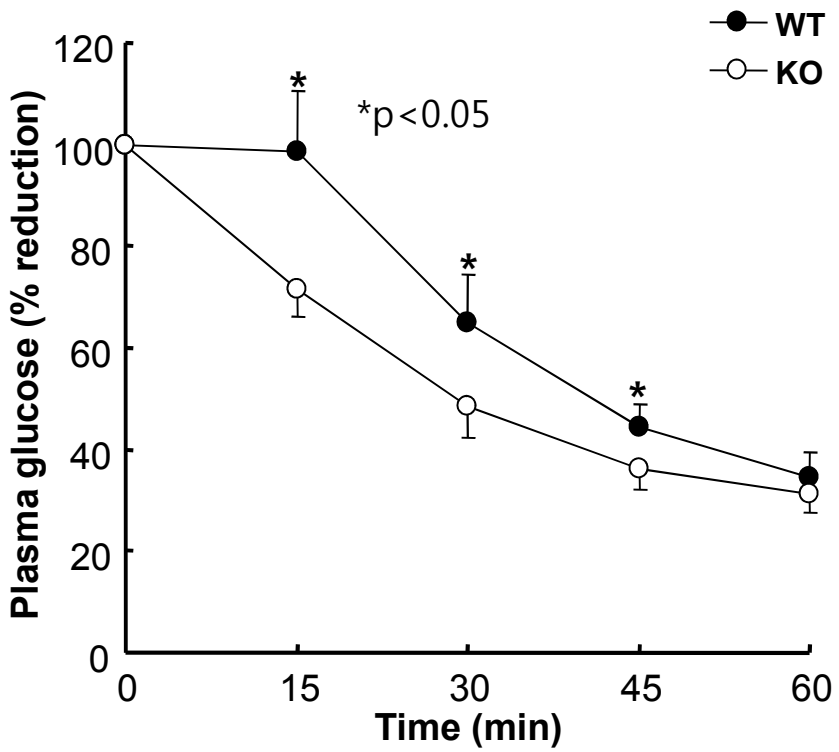
Mouse soleus muscle

IRS-1 expression level is elevated in TRIM-disrupted skeletal muscle



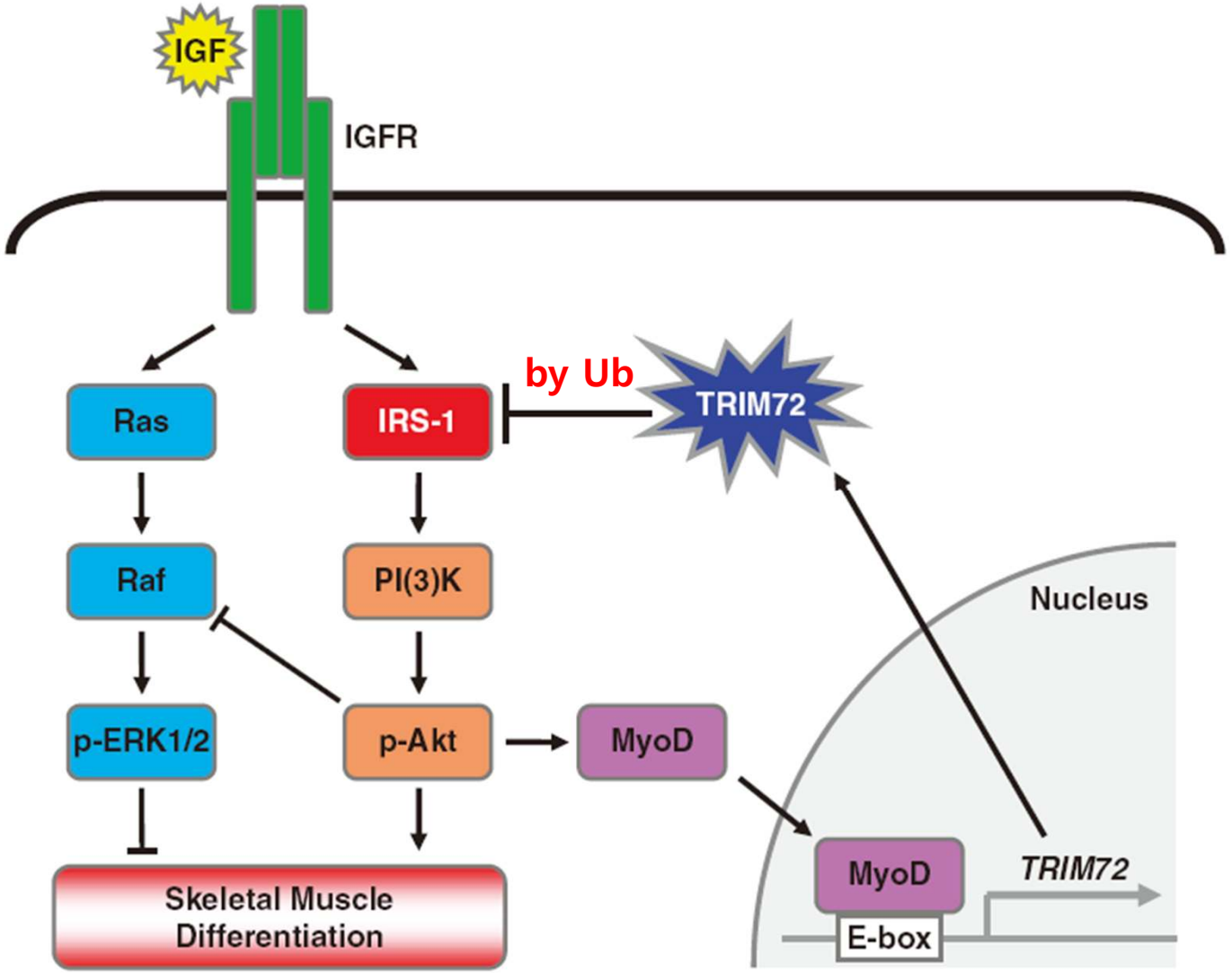
TRIM72 is a therapeutic target for type 2 diabetes





High fat diet-fed mice

E3 ligase TRIM72 negatively regulates myogenesis by IRS-1 ubiquitination.



Suggestion; TRIM72 is a negative muscle size regulator.

How do organs and whole organisms know when to stop growing?
A person's right and left legs almost always end up the same length, and the hearts of mice and elephants each fit the proper rib cage. How genes set limits on cell size and number continues to mystify.

JUPITER IMAGES

Korea University

Lab of Cell Signal Transduction

Dr. Jae-Sung Yi
Dr. Chang-Seok Lee
Dr. Young-Mi Ham
Miss Na-Rae Lee
Ms. Nga Nguyen
Mr. Jing Hong
Mr. June-Seop Lee
Mr. Jeong-Woo Lee
Mr. Dong-Min Yoo