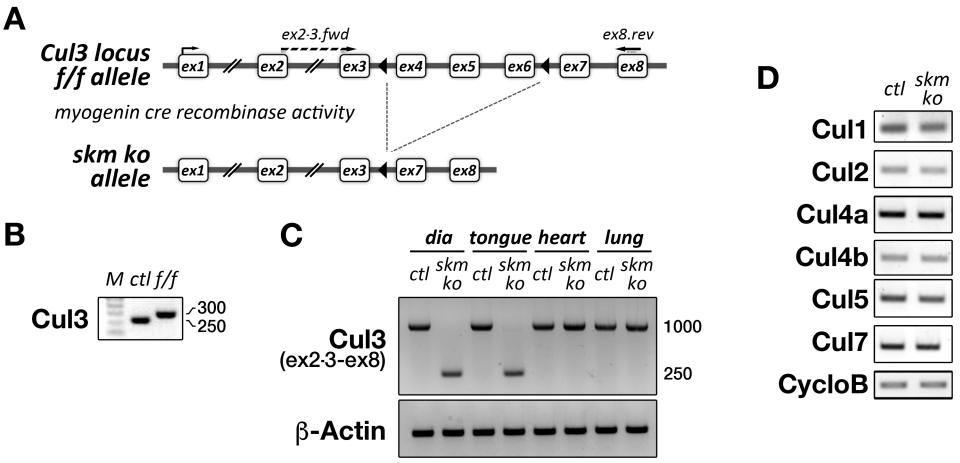
Supplementary Figures, Figure Legends and Tables



Supplementary Figure 1. Generation and validation of *skm-KO* animals.

(**A**) Schematic representation of the strategy used to specifically delete *Cullin-3* in skeletal muscles. (**B**) Result of PCR done on genomic DNA used to genotype Cullin-3 floxed animals. M: molecular-weight marker; *ctl*: control; *f/f*: flox/flox (**C**) RT-PCR on Cullin-3 mRNA using ex2-3.fwd and ex8.rev primers in various tissues showing a recombined transcript only in E18.5 skeletal muscles of *skm-KO*. dia: diaphragm. (**D**) Semi-quantitative RT-PCR of Cullin mRNA levels in E18.5 diaphragms of *ctl* and *skm-KO*.

A

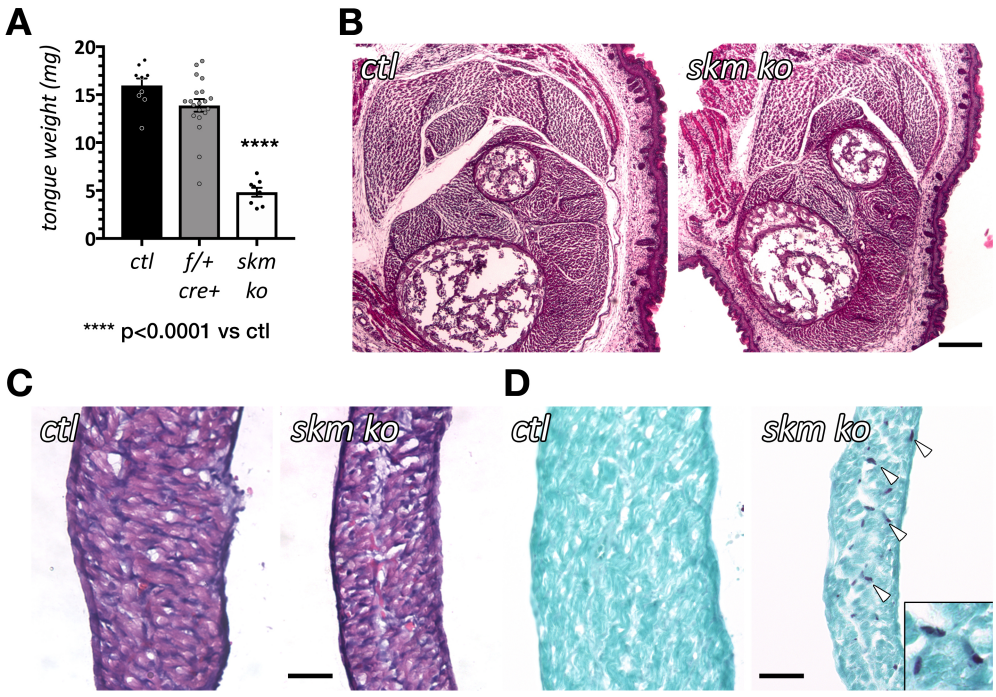
Stage	*ctl*	*f/+ cre+*	*skm ko*
E18.5	*24 (31%)*	*35 (45%)*	*18 (24%)*
P21	*23 (41%)*	*33 (59%)*	*0 (0%)*

B

cyanotic embryos	
ctl	*0/18 (0%)*
skm ko	*13/13 (100%)*

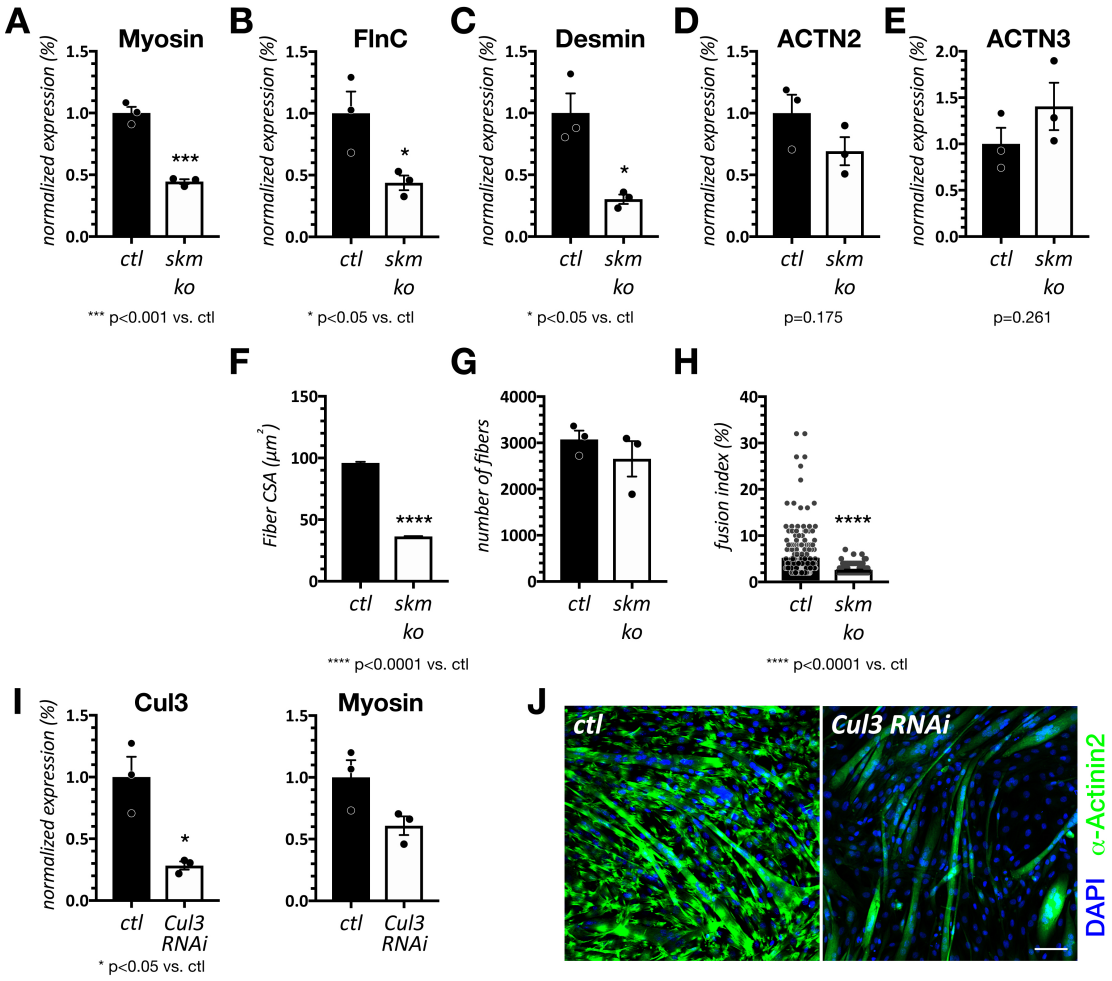
Supplementary Figure 2. Early developmental loss of skeletal muscle Cullin-3 leads to postnatal death and respiratory defects.

(**A**) Breeding outcome at E18.5 and weaning stages. Number of analyzed animals and corresponding percentages (in brackets) for each genotype are indicated. (**B**) Quantification of the cyanotic embryos 5 minutes after delivery (n=18 for *ctl* and n=13 for *skm-KO*).



Supplementary Figure 3. Absence of Cullin-3 leads to severe skeletal muscle myopathy.

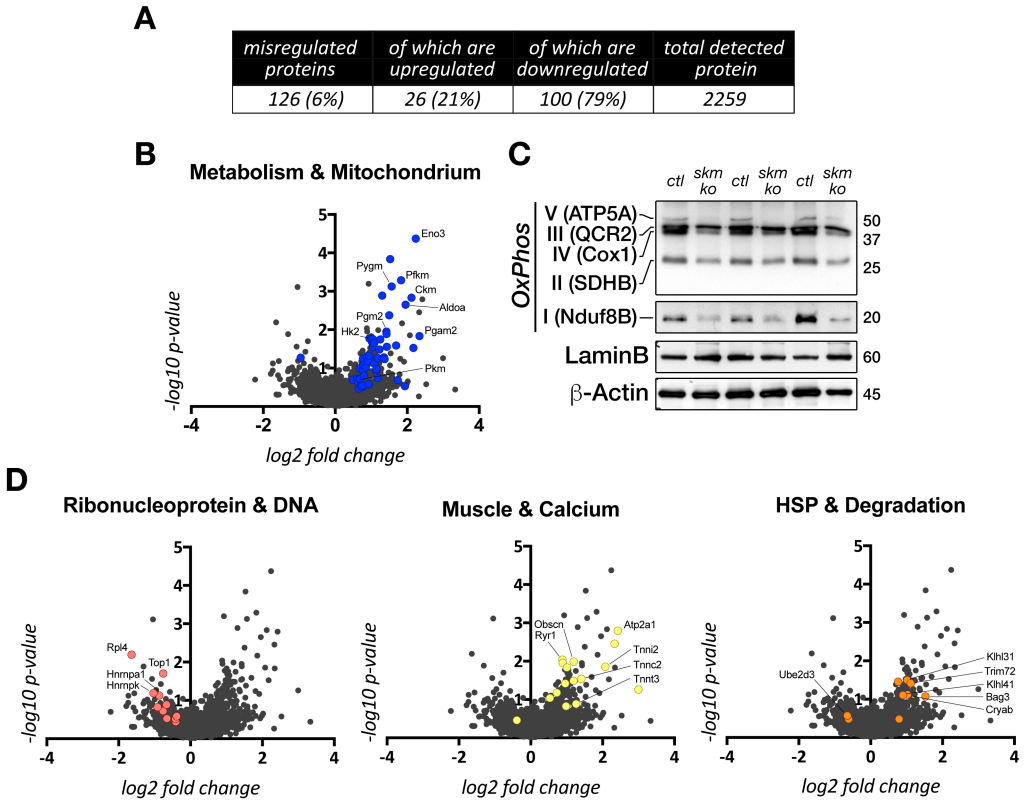
(**A**) Analysis of tongue weights revealing a strong muscle atrophy in E18.5 *skm-KO*. (n=9 for *ctl*, n=20 for heterozygous (*f/+;cre+*) and n=8 for *skm-KO*). ***$P<0.0001$ by Anova and Bonferroni's multiple comparisons test. (**B**) Cross-sections of whole hindlimbs stained with Hematoxylin-Eosin, indicating muscle atrophy in *skm-KO*. Scale bar = 200µm. (**C-D**) Cross-sections of E18.5 diaphragms stained with Hematoxylin-Eosin (**C**) or modified Gomori trichrome (**D**) reveal aggregates in *skm-KO*. Arrowheads indicate accumulated material. Scale bar = 100µm for C and D.



Supplementary Figure 4. Loss of Cullin-3 leads to muscle fiber hypotrophy and maturation defects.

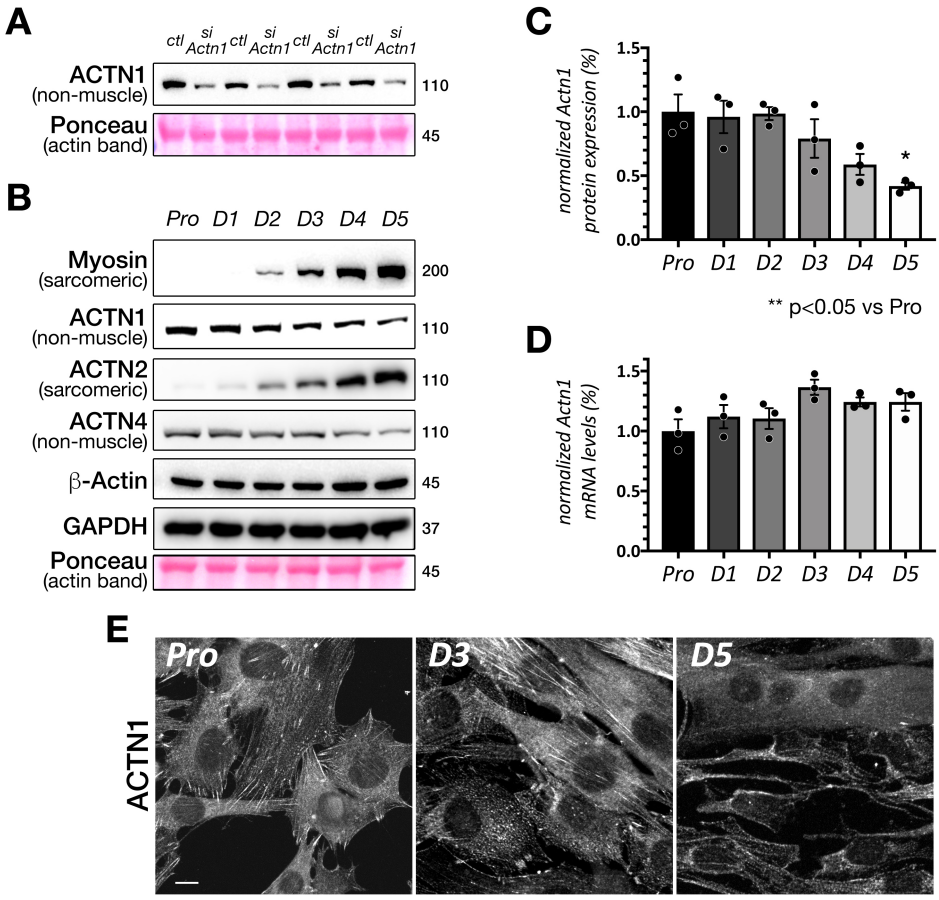
(**A-C**) Quantification of (**A**) sarcomeric Myosin, (**B**) Filamin-C (FlnC) and (**C**) Desmin protein levels showing a decrease in the expression of muscle maturation markers in E18.5 diaphragms of *skm-KO* (n=3 for each genotype). (**D-E**) Quantification of sarcomeric ACTN2 (**D**) and ACTN3 (**E**). n=3 for each genotype. *$P<0.05$, ***$P<0.001$ by two-tailed t-test. (**F**) Average cross-sectional area (CSA, in μm^2) of fibers showing hypotrophy in *skm-KO* (n=3 for each genotype). ****$P<0.0001$ by two-tailed t-test. (**G**) Average number of fibers that constitute E18.5 diaphragms of *ctl* and *skm-KO* (n=3 for

each genotype). P=0.378 by two-tailed t-test. (**H**) Quantification of the fusion index of *skm-KO* satellite cells and controls, revealing a strong decrease in the number of nuclei per myotube after three days of differentiation. (n ≥ 182 per group). ****$P<0.0001$ by two-tailed t-test. (**I**) Quantifications of Cullin-3 and Myosin protein levels in C2C12 myotubes transfected with *Cullin-3* or *scramble siRNA* after 5 days of differentiation (n=3 for each condition). *$P<0.05$ by two-tailed t-test. (**J**) Immunofluorescence staining of C2C12 myotubes differentiated for 5 days and transfected with a *Cullin-3* (siCul3) or a *scramble siRNA.* Cells were labeled with sarcomeric ACTN2 antibody and DAPI. Scale bar = 100µm.



Supplementary Figure 5. Loss of Cullin-3 in skeletal muscles leads to deregulation of proteins involved in metabolism, mitochondria, calcium-handling, ribonucleoprotein and DNA as well as proteins involved in heat-shock and degradation. (**A**) Results of the proteome analysis performed on E18.5 diaphragms of *skm-KO* compared to *ctl*. Total numbers of significantly deregulated proteins are shown. (**B**) Volcano plot of significantly deregulated proteins in diaphragms of *skm-KO* that are involved in metabolism and mitochondria. (**C**) Immunoblot analysis demonstrating decreases for several mitochondrial proteins (using the OxPhos antibody cocktail) in diaphragms of *skm-KO* compared to controls. (**D**) Volcano plot of proteins that are significantly

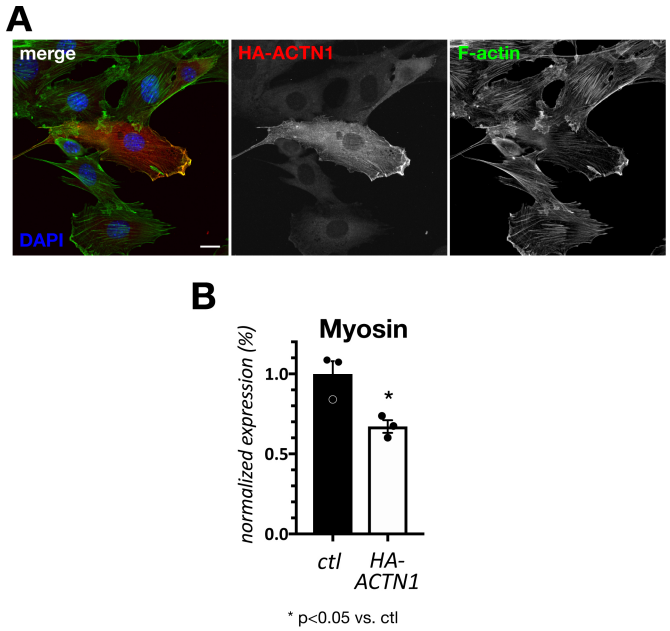
deregulated in diaphragms of *skm-KO* and that are associated with Ribonucleoproteins or DNA, muscle and calcium handling, or heat shock (HSP) and protein degradation.



Supplementary Figure 6. ACTN1 protein levels are decreased during normal muscle differentiation.

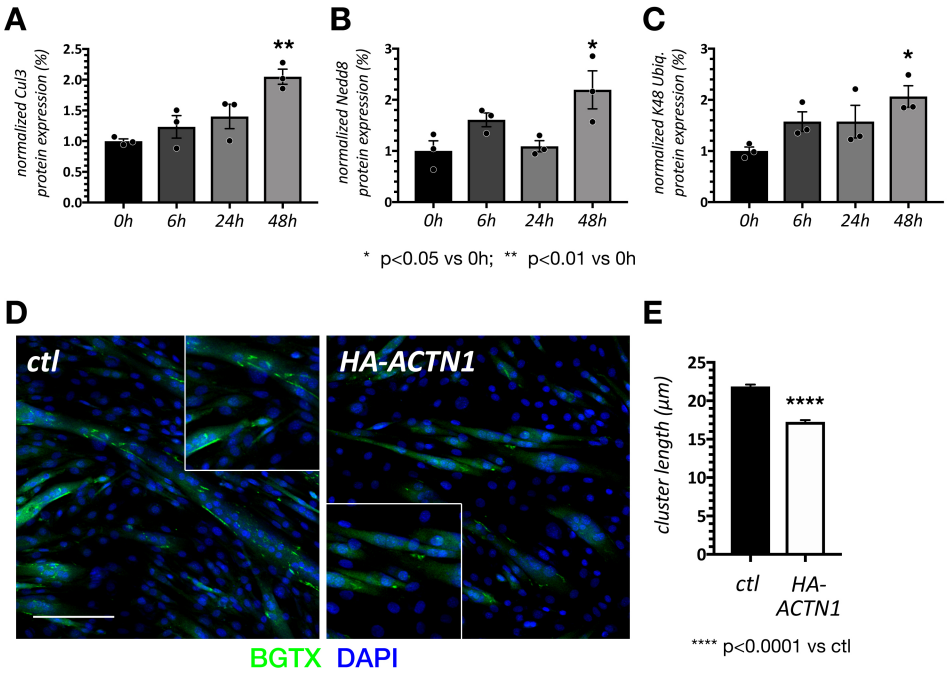
(**A**) Validation of ACTN1 antibody by immunoblot analyses using C2C12 cells expressing *Actn1 siRNA* (si Actn1). (**B**) Immunoblots of alpha-Actinin protein isoforms in C2C12 revealing a decreased expression of non-muscle actinins (ACTN1 and 4) and increased expression of the muscle ACTN2. (**C-D**) Analysis of ACTN1 protein (C) and mRNA (D) levels in C2C12 over 5 days of differentiation (n=3 per time point). (n=3 per time point) **P<0.05* by Anova and Bonferroni's multiple comparisons test. (B-D) *Pro*: proliferation; *D1-5*: differentiation day 1 to 5. (**E**) Immunofluorescence staining of C2C12 cells in

proliferation (*Pro*) or after 3 (*D3*) and 5 days (*D5*) of differentiation with an antibody against ACTN1. Scale bar = 10μm.



Supplementary Figure 7. Over-expression of ACTN1 in C2C12.

(**A**) C2C12 over-expressing HA-ACTN1 and stained with HA antibody (red), Phalloidin (green) and DAPI (blue) showing correct localization of the tagged protein. Scale bar = 10µm. (**B**) Quantification of Myosin protein expression in C2C12 expressing HA or HA-ACTN1 constructs after 5 days of differentiation (n=3 per condition). **P<0.05* by two-tailed t-test.



Supplementary Figure 8. Regulation of Cullin-3, Cullin activity and ACTN1 are important for normal AchR clustering. (**A**) Quantification of Cullin-3 (Cul3) protein levels in C2C12 myotubes following neural Agrin stimulation, indicating a marked increase after 48 hours (n=3 per time point). ***P<0.01*, by Anova and Bonferroni's multiple comparisons test. (**B**) Quantification of Nedd8-linked protein levels (measured at 80kDa) in C2C12 myotubes following neural Agrin stimulation, showing a marked increase in neddylated-Cullin proteins after 48 hours (n=3 per time point). **P<0.05* by Anova and Bonferroni's multiple comparisons test. (**C**) Quantification of poly-ubiquitylated protein levels in C2C12 myotubes following neural Agrin stimulation, showing a marked increase in K48-ubiquitylated proteins after 48 hours. (n=3 per time point). **P<0.05* by Anova and Bonferroni's multiple comparisons test. (**D**) Immunofluorescence staining of AchR clusters in C2C12 myotubes expressing HA-ACTN1

or HA-control constructs with fluorescent-Bungarotoxin (BGTX) after 48 hours. Scale bar = 100µm. (**E**) Quantification of AchR cluster-sizes in C2C12 myotubes expressing HA-control or HA-ACTN1 constructs after 48 hours of stimulation with neural Agrin (n=3 per condition). *****P<0.0001*, by two-tailed t-test.

Supplementary Table 1. Oligonucleotides for RT-PCR and RT-qPCR

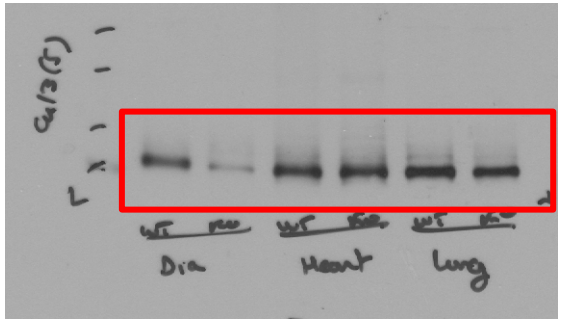
Gene names	Oligonucleotide names	Sequences
Cullin-1	Cul1.fwd	GAATAAACAGGTAACAAATG
	Cul1.rev	ATTCTTTATACACTGTTAACG
Cullin-2	Cul2.fwd	GAGCTAGCATTGGATATGTGG
	Cul2.rev	ATGGATTACTTTCTGGTTTGG
Cullin-3	Cul3.fwd	GACTATATCCAGGGCTTATTG
	Cul3.rev	TGAGAGGTATTCAGGAGACC
Cullin-4A	Cul4A.fwd	CTCCAGGTGTACAAAGACTC
	Cul4A.rev	CGGTTTTTGTGTGCTGTGGTC
Cullin-4B	Cul4B.fwd	CTACCAGGCTGTAGAAAATC
	Cul4B.rev	GGTTTTGCCAGCATCTATCG
Cullin-5	Cul5.fwd	GAGTGGCTAAGAGAAGTTGG
	Cul5.rev	CGGAATCAGCTGGTAATGCC
Cullin-7	Cul7.fwd	GCTGGGACCCGGACCCAGAT
	Cul7.rev	CTGGGACCTGTGGCAGCTG
ACTN1	Actn1.fwd	GCCAGGACCATCAATGAAGT
	Actn1.rev	GAACTCTTCGGGACCCAAC
cyclophilin B	cycloB.fwd	GATGGCACAGGAGGAAAGAG
	cycloB.rev	AACTTTGCCGAAAACCACAT

Supplementary Table 2. List of antibodies.

Antigen	Antibody reference/Clone number	Manufacturer, comments
Alpha-Actinin 1 (ACTN1)	sc-135819, clone 23	Santa Cruz Biotechnology
Alpha-Actinin 1 (ACTN1)	Home-made, clone 3A2	Courtesy of Dr. Beggs, Children's Hospital Boston, Massachusetts, USA
Alpha-Actinin 2 ACTN2	EA-53	VWR
Alpha-Actinin 2 ACTN2	Home-made, clone 4A3	Courtesy of Dr. Beggs, Children's Hospital Boston, Massachusetts, USA
Alpha-Actinin 3 ACTN3	ab68204 (EP2531Y)	Abcam
Alpha-Actinin 4 ACTN4	ab108198	Abcam
Alpha-Actinin 4 ACTN4	Home-made, clone 6A2	Courtesy of Dr. Beggs, Children's Hospital Boston, Massachusetts, USA
Beta-Actin	sc-47778	Santa Cruz Biotechnology
Cullin-1	C7117	Sigma-Aldrich
Cullin-3	Home-made	Generated in the laboratory of Dr. Singer (21)
Desmin	sc-14026	Santa Cruz Biotechnology
Filamin-C	NBP1-89300	Novus Biologicals
GAPDH	sc-32233	Santa Cruz Biotechnology
HA	3F10	Roche
KBTBD13	LS-C166810	LS Bio
KBTBD5	PA5-23933	Thermo Scientific
KCTD6	ab62596	Abcam
KCTD9	SAB1105110	Sigma-Aldrich
KLHL9	PA5-25097	Thermo Scientific
Lamin B1	#12586 (D4Q4Z)	Cell Signaling Technology
Myogenin	F5D	DSHB (deposited by Wright, W.E.)
Myomesin-3 Myom3	Home-made	Kind gift of Dr. Agarkova (87)
Nedd8	#2754	Cell Signaling Technology
Neurofilament	ab1991	Millipore
OxPhos	458099	Novex
p62	GP62-C	Progen
Sarcomeric Myosin Heavy Chain	A4.1025	DSHB (deposited by Blau, H.M.)
PLZF	ab39354	Abcam
Porin/VDAC1	ab14734 (20B12AF2)	Abcam
Synaptophysin	H-93	Santa Cruz Biotechnology
Ubiquitin-K48	#8081 (D9D5)	Cell Signaling Technology

Figure 1A

Cul3



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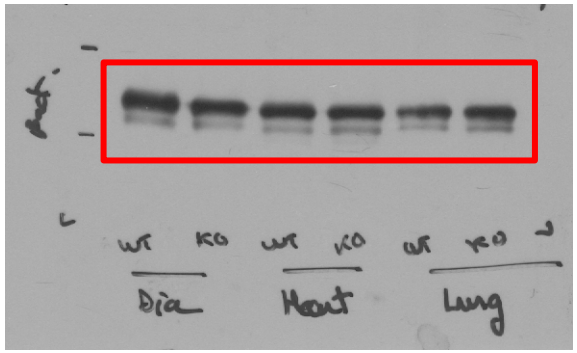
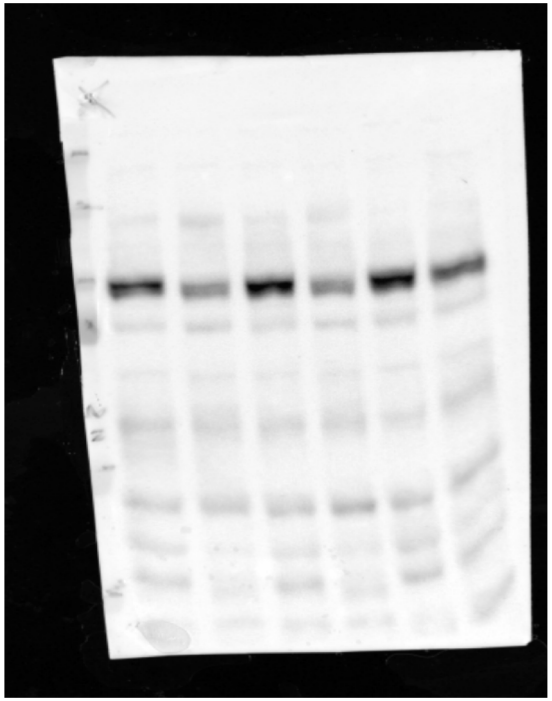


Figure 1C

NEDD8



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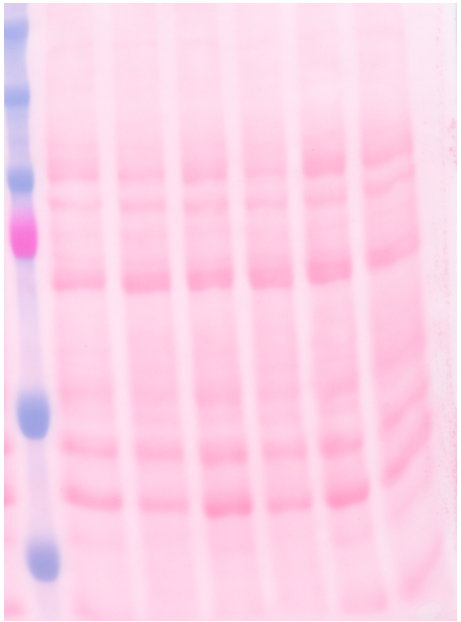
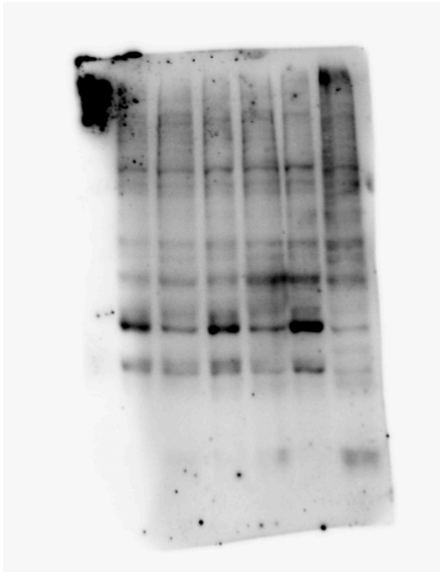
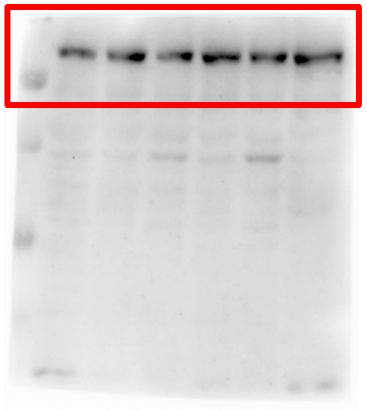


Figure 1E

Ubiq. K48



P62



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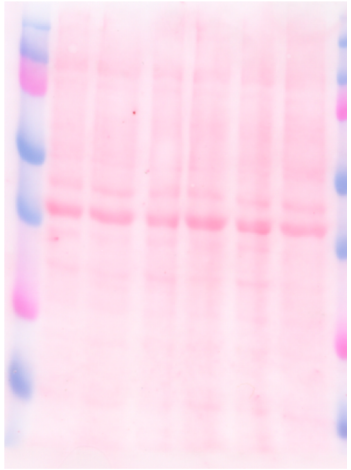


Original Ponceau (not shown In figure)

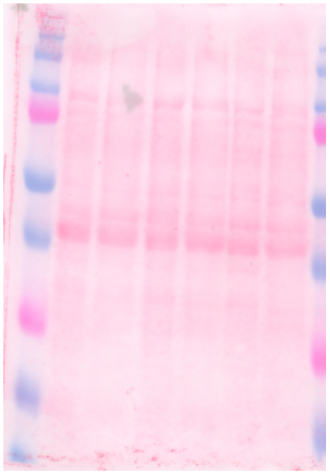
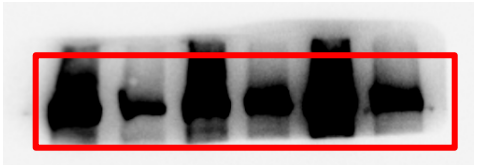
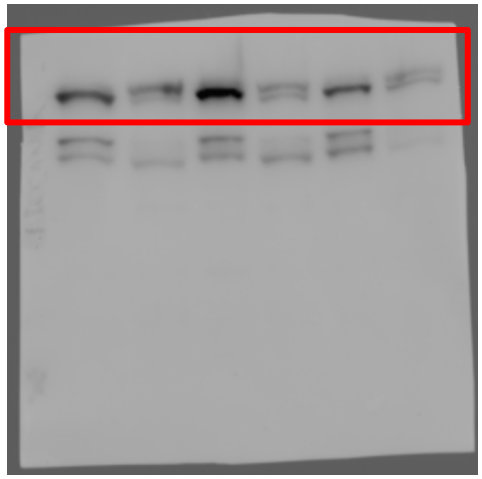
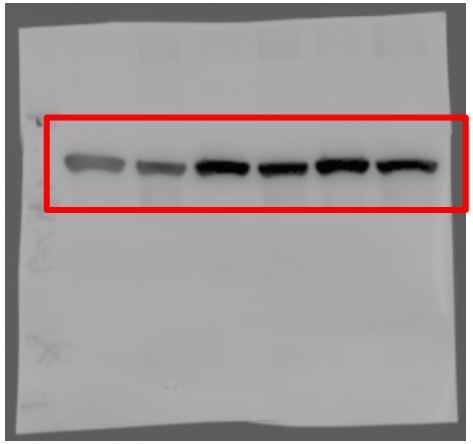


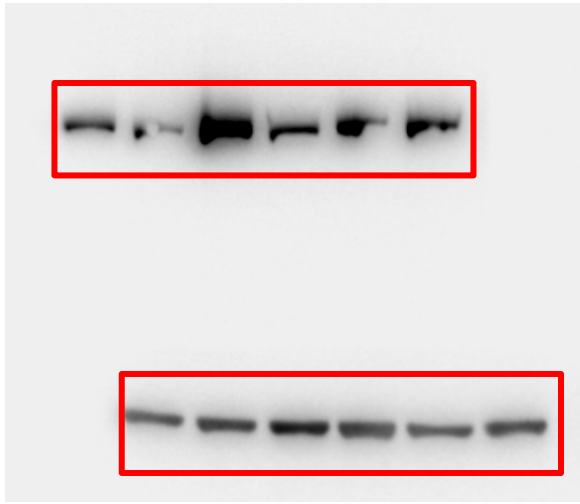
Figure 3D


Myosin Heavy Chain


Filamin-C


Desmin


Beta-Actin


ACTN2
Beta-Actin

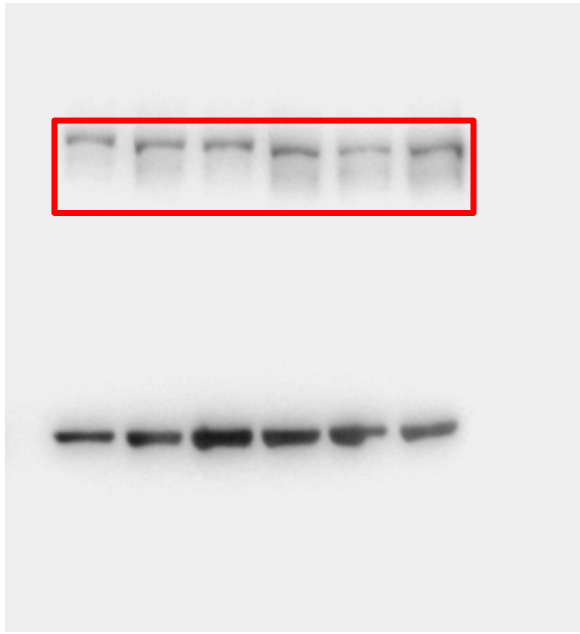

ACTN3
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Figure 4C: DNA gel

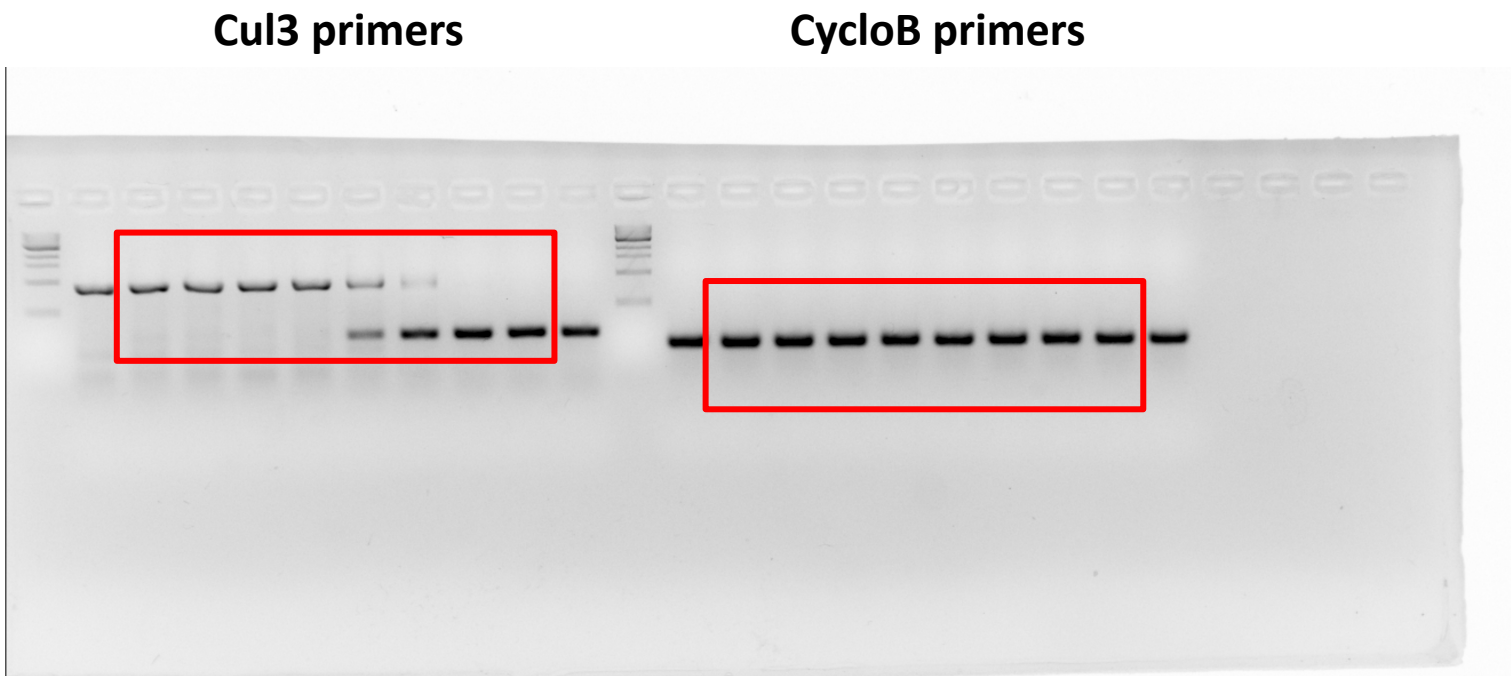


Figure 4E

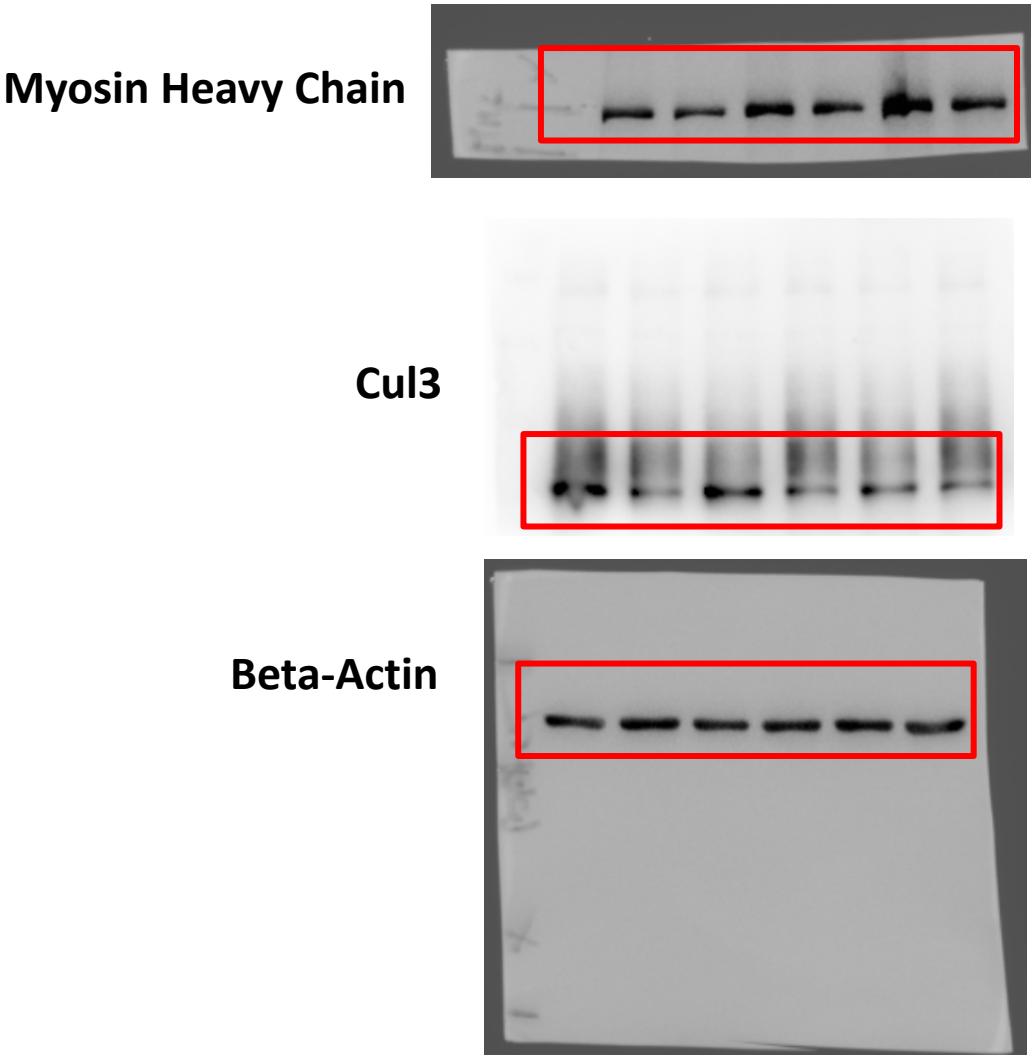


Figure 5A (1/2)

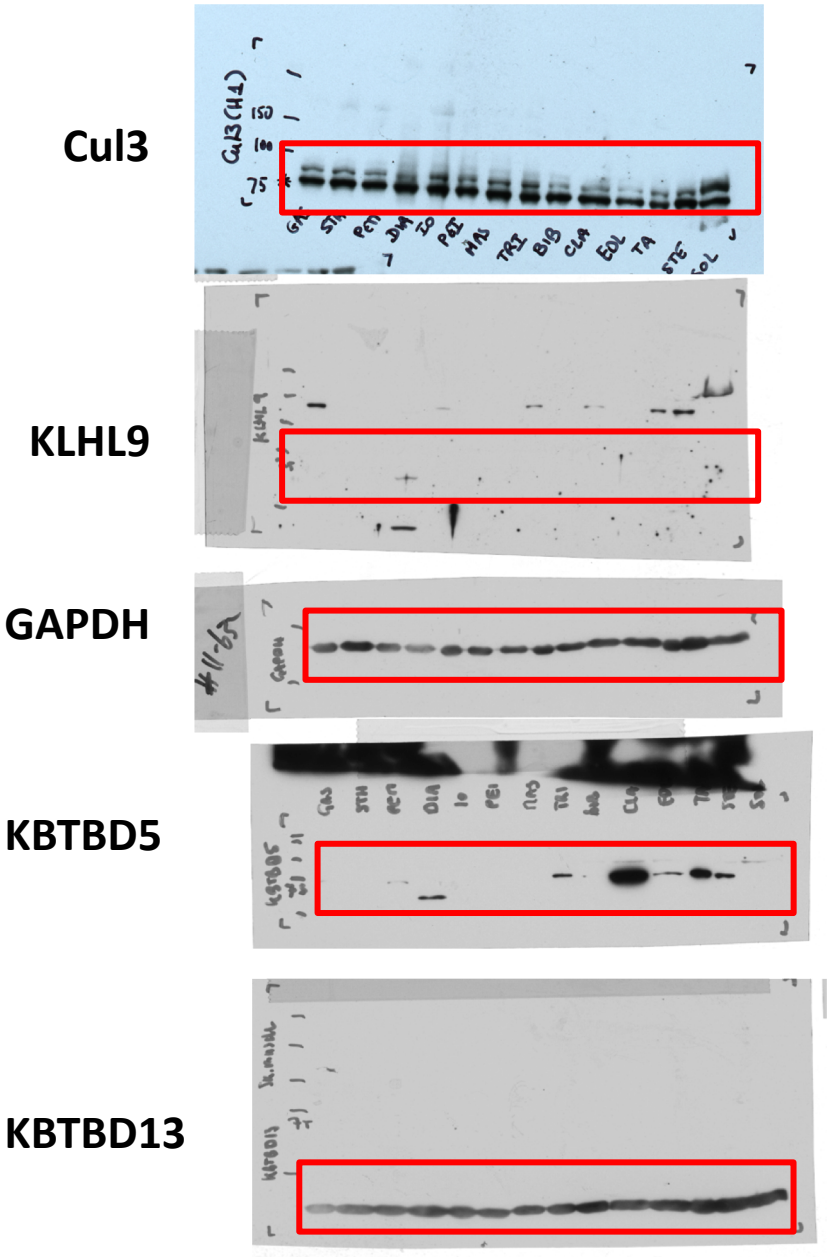


Figure 5A (2/2)

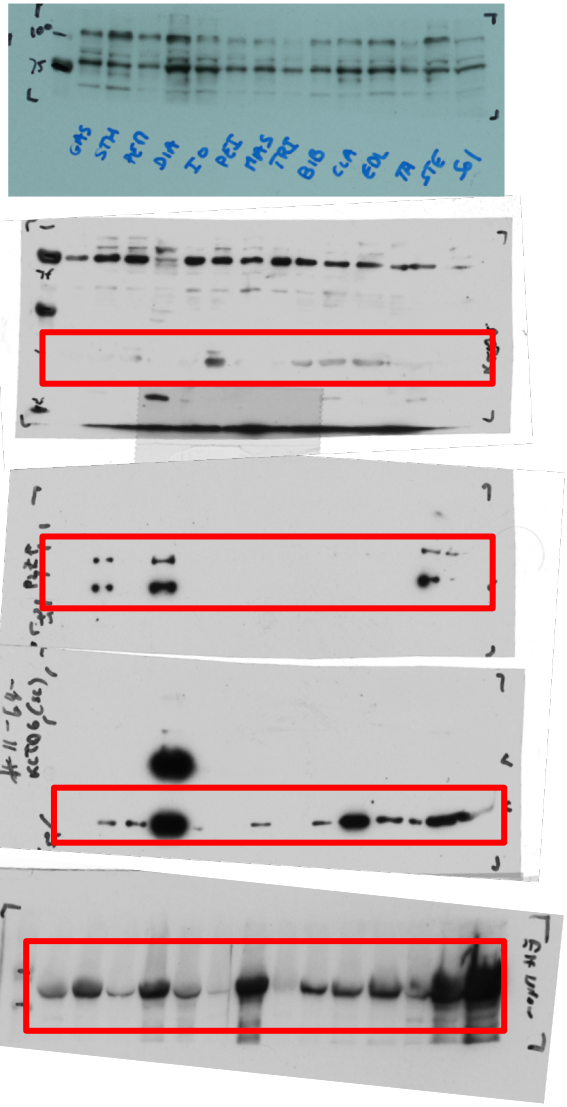
Cul1

KCTD9

PLZF

KCTD6

Myom3



Silverstain
Myosin

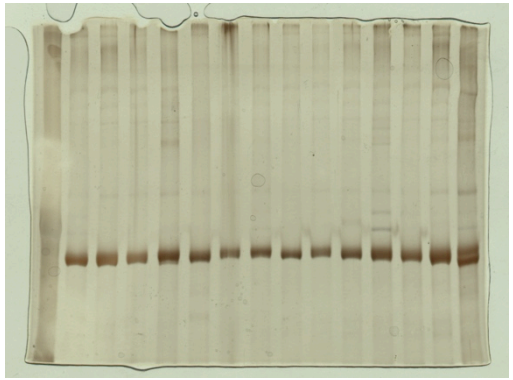
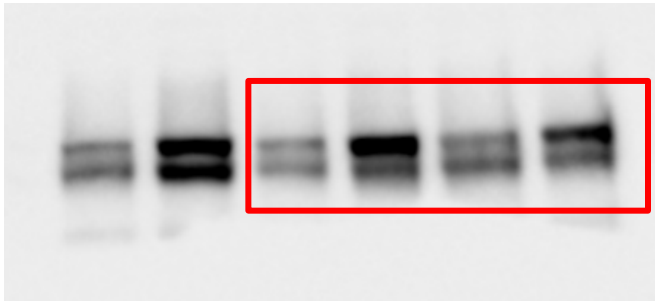


Figure 6A

ACTN1



Beta-Actin

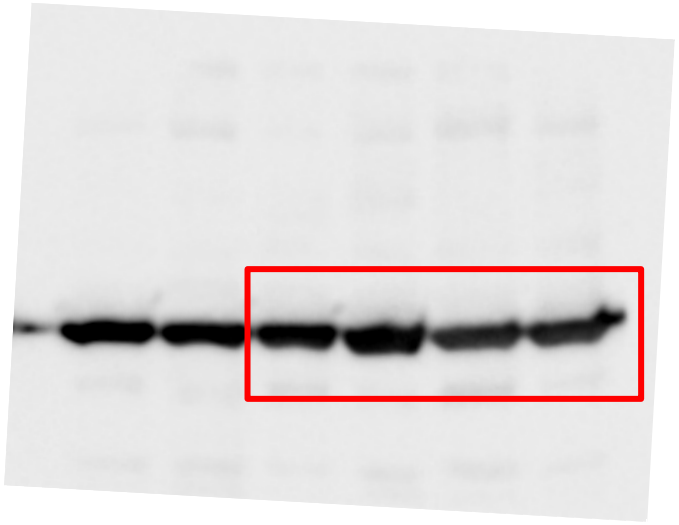


Figure 6C

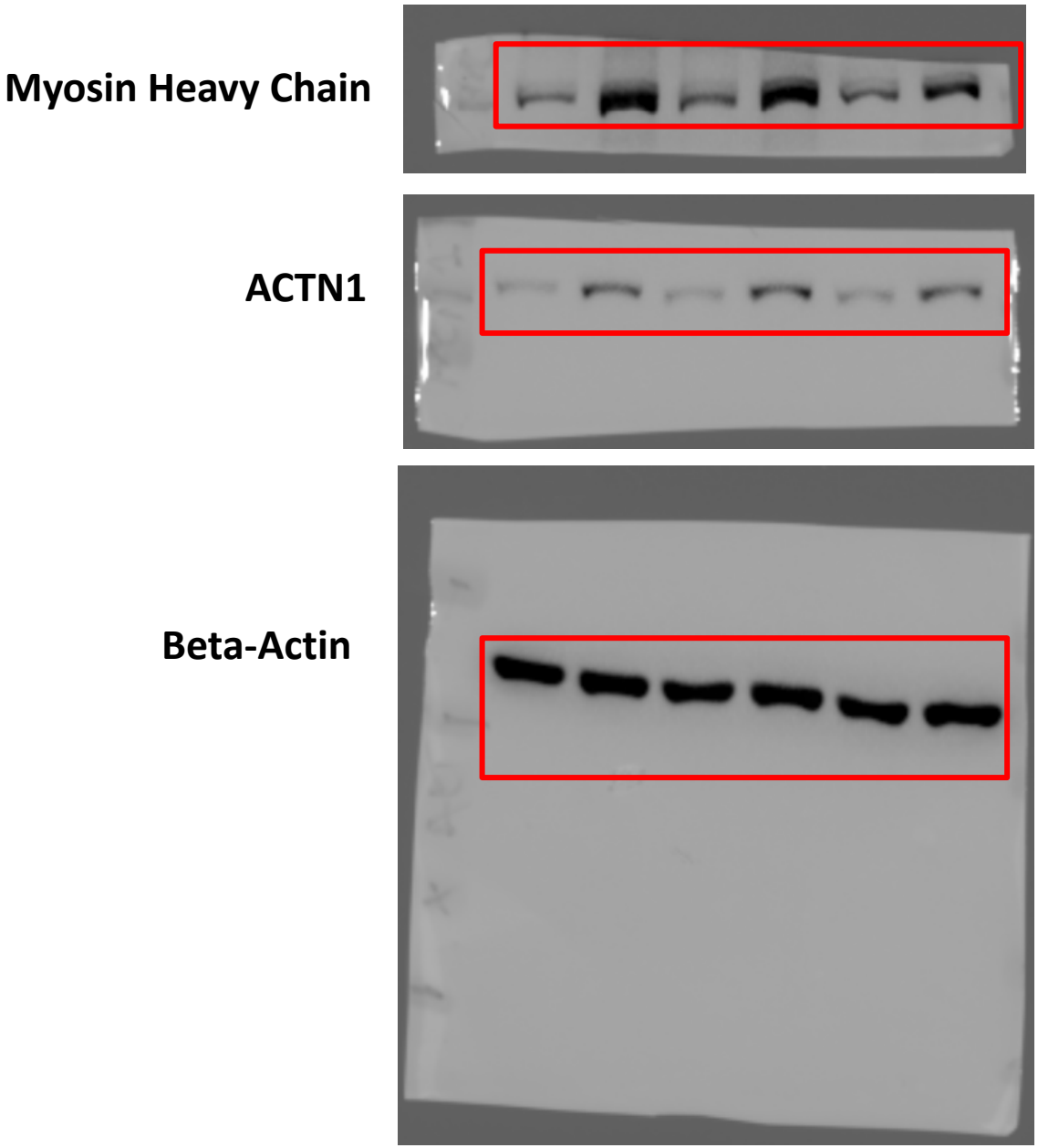
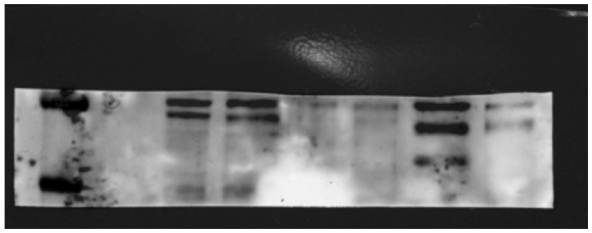
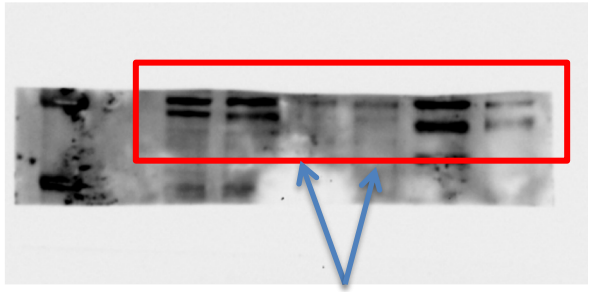


Figure 6E





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original ponceau stain of gel

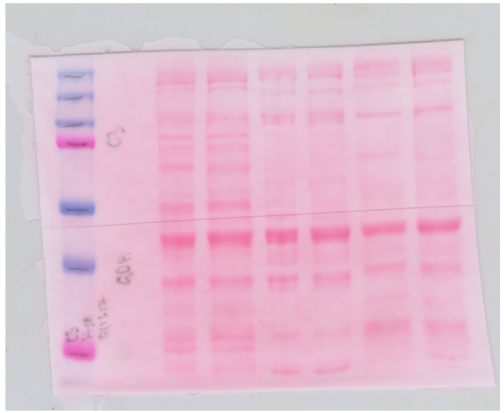
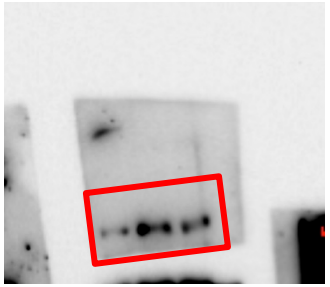
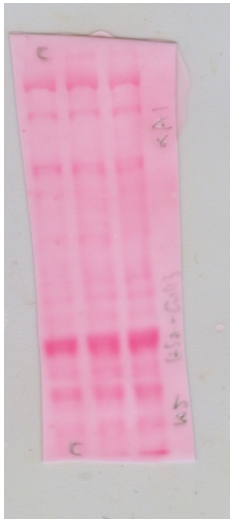


Figure 6F



ACTN1 blot
and original ponceau
stain of membrane



ACTN2 and GAPDH blots
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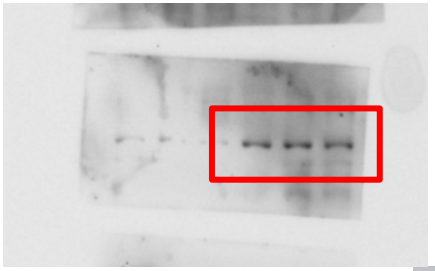
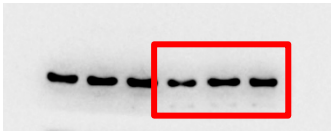
stain of membrane





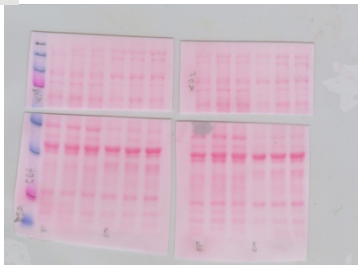


Figure 7A

Myosin Heavy Chain

HA-ACTN1

GAPDH

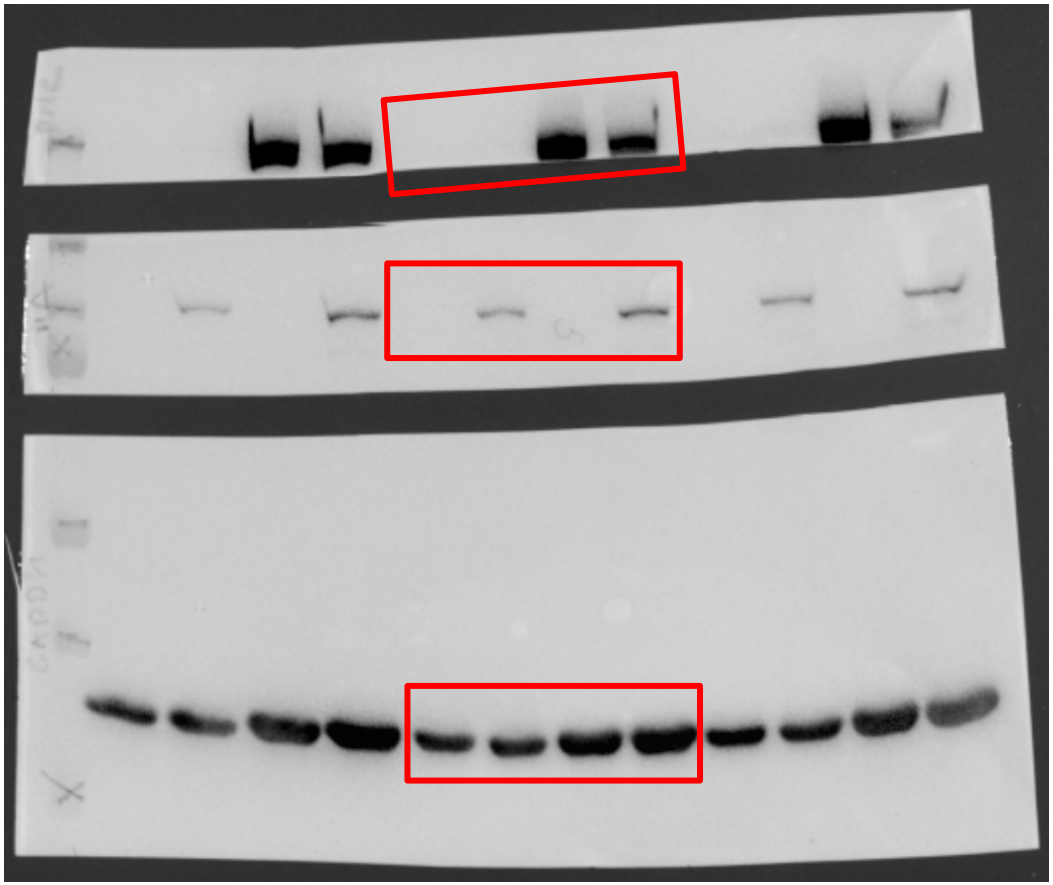


Figure 8A

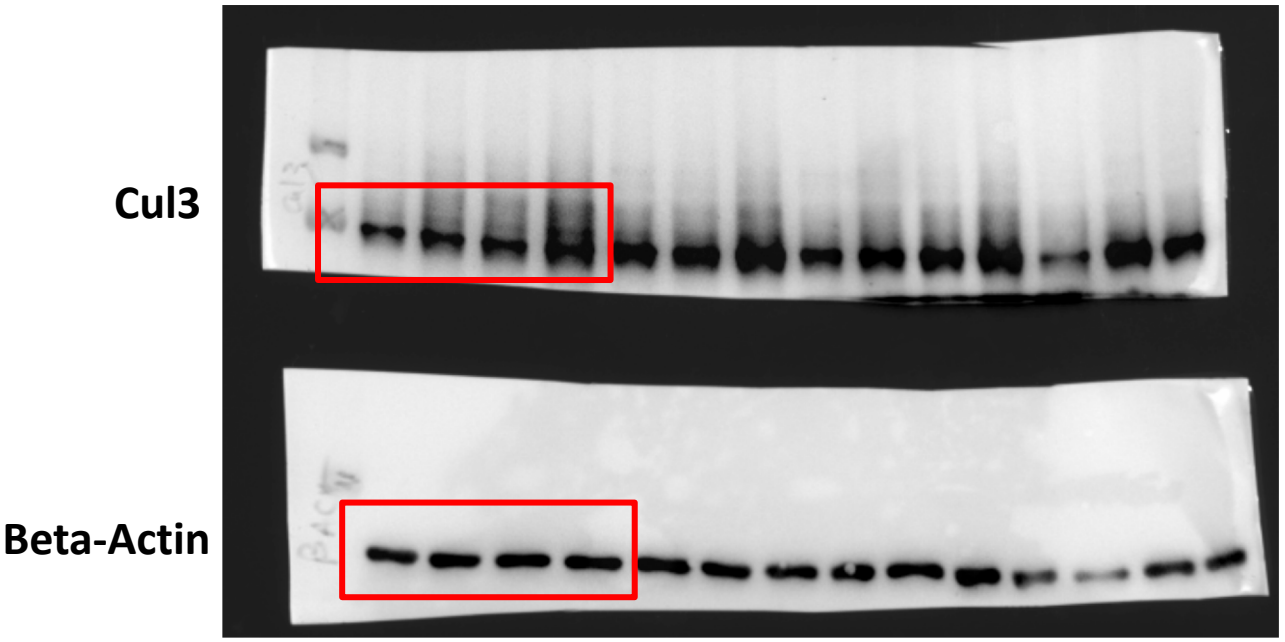
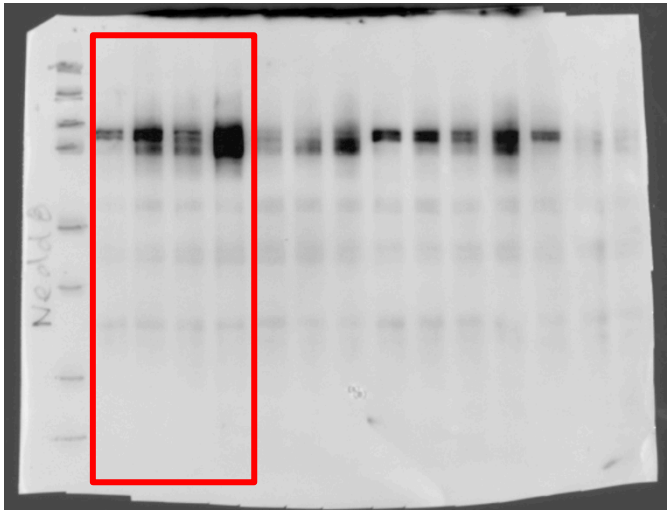


Figure 8B

NEDD8



Original Ponceau

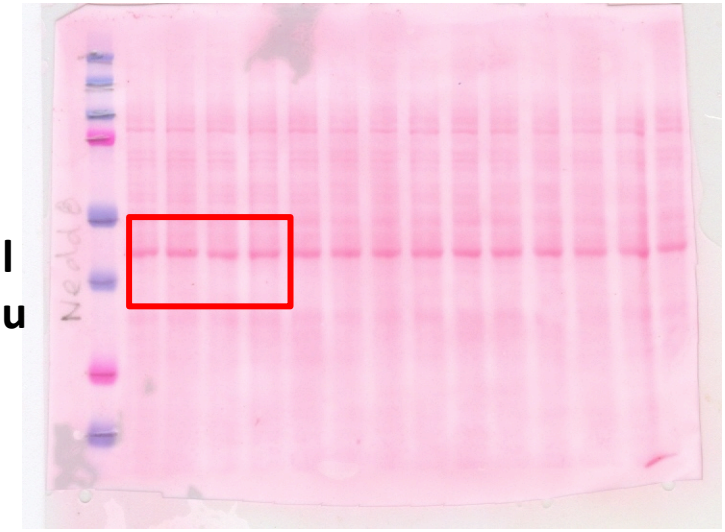
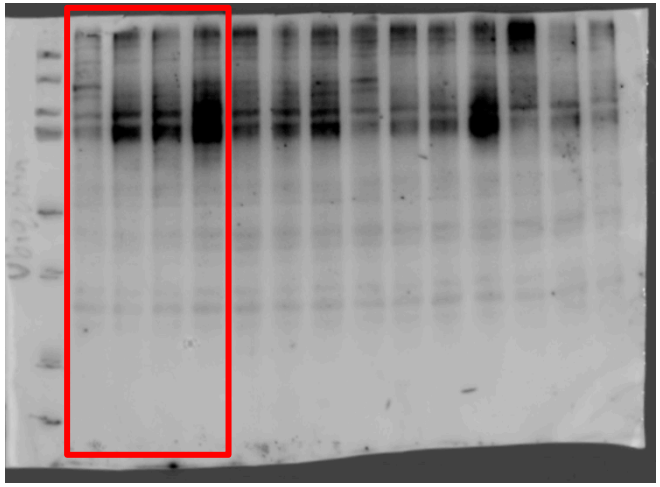


Figure 8C

Ubiq (K48)



Original Ponceau

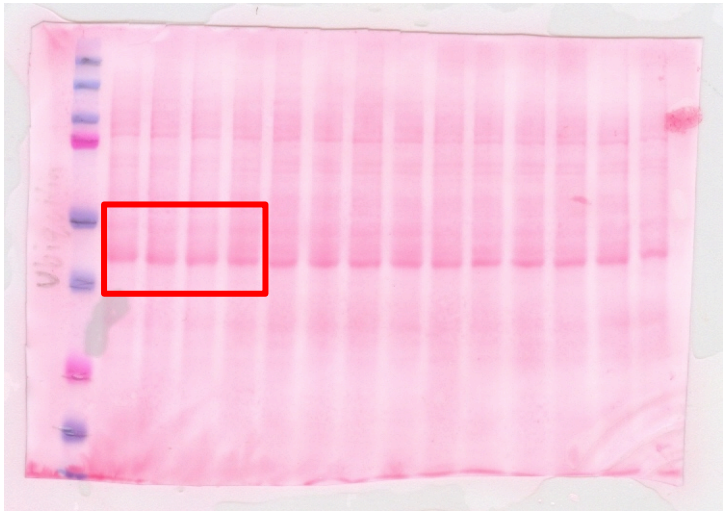
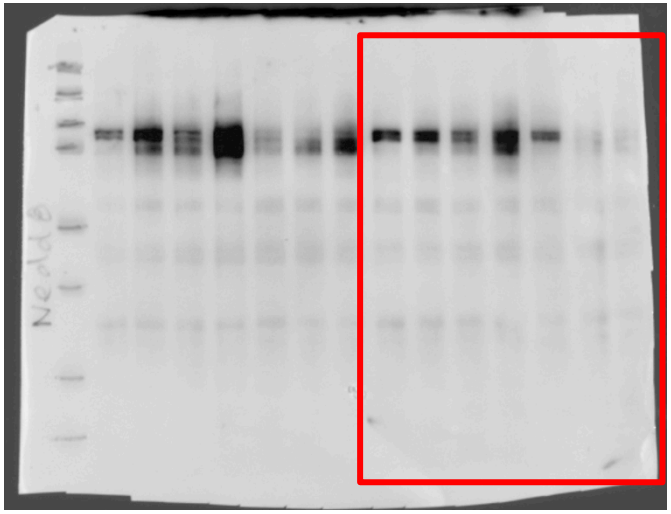


Figure 8D

NEDD8



Original Ponceau

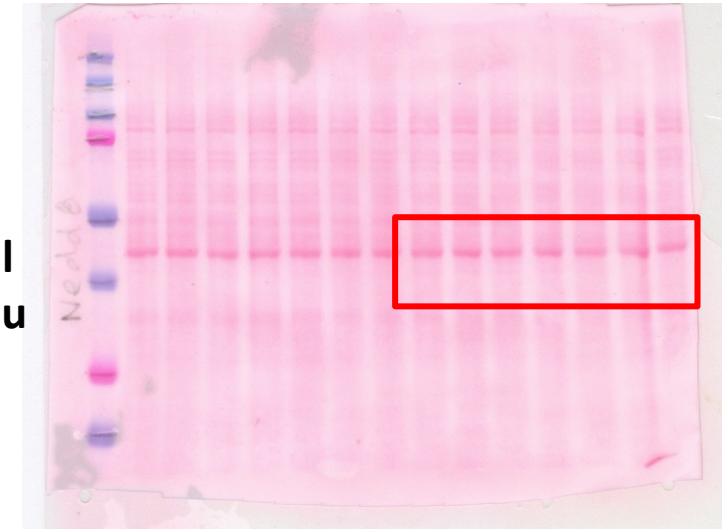
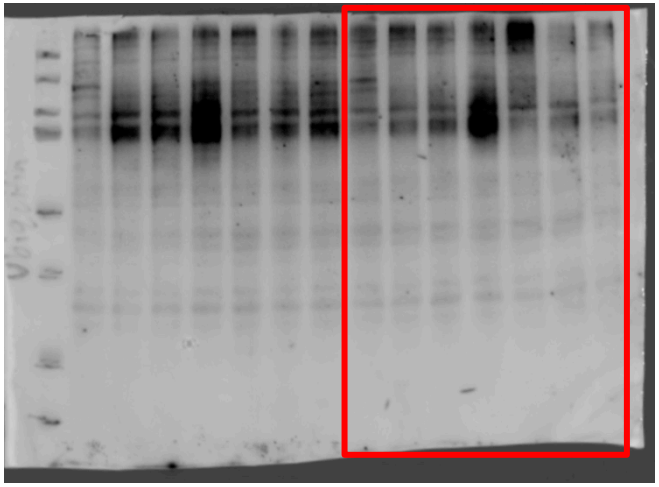


Figure 8E

Ubiq (K48)



Original Ponceau

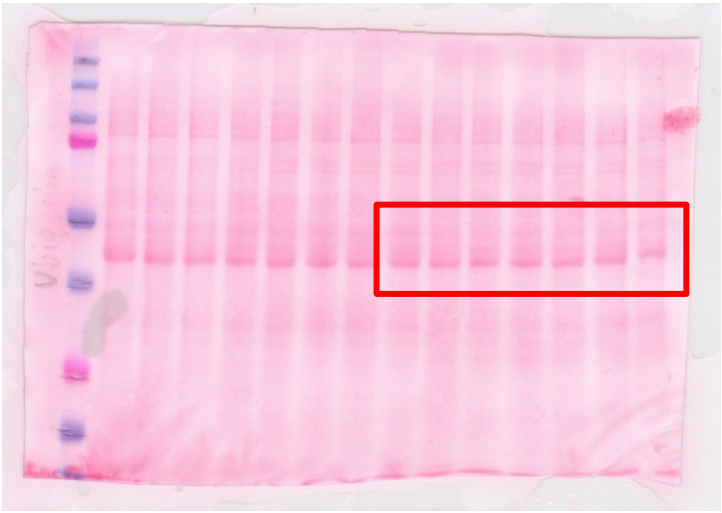


Figure 10B (1/2)

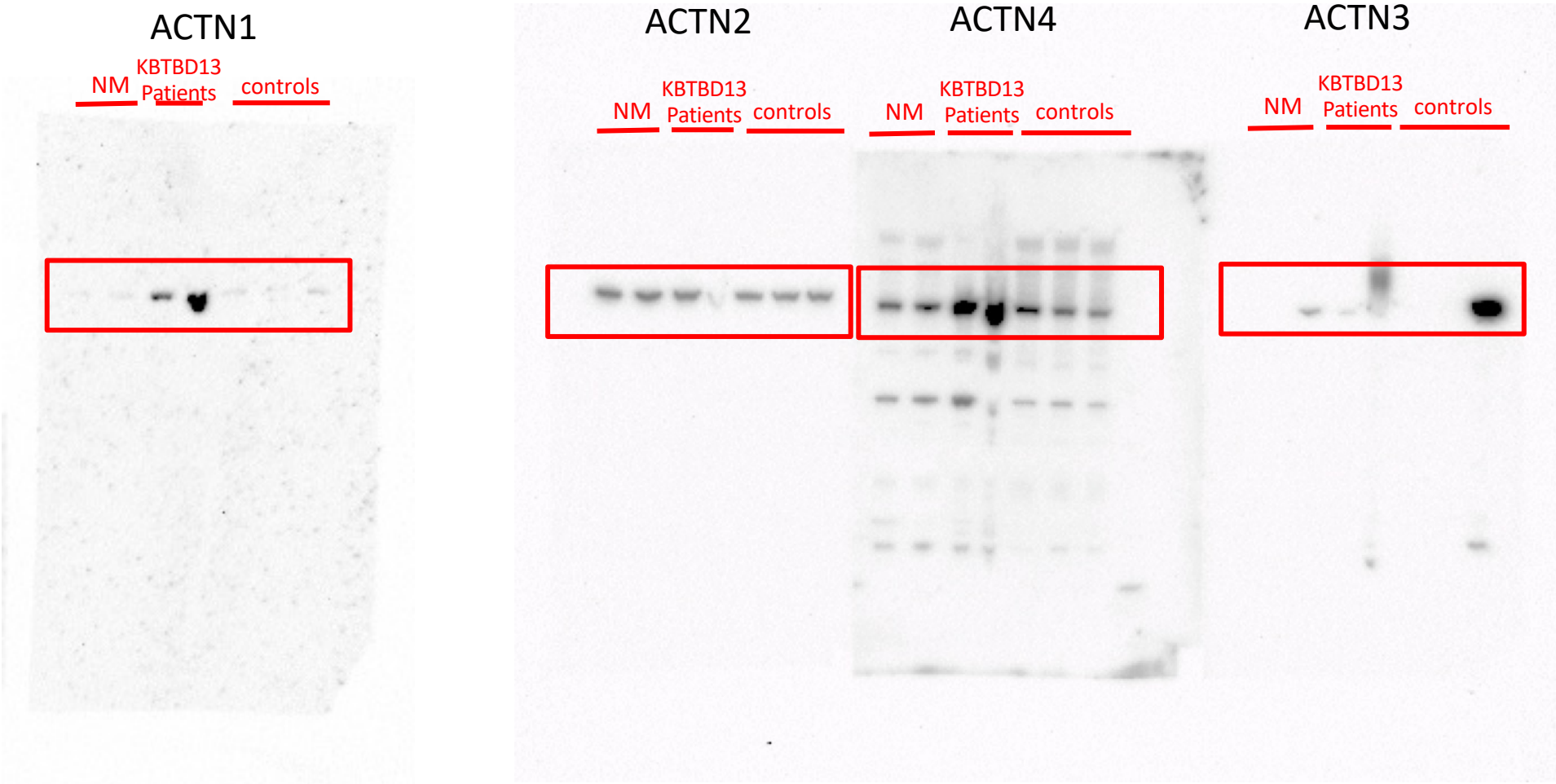
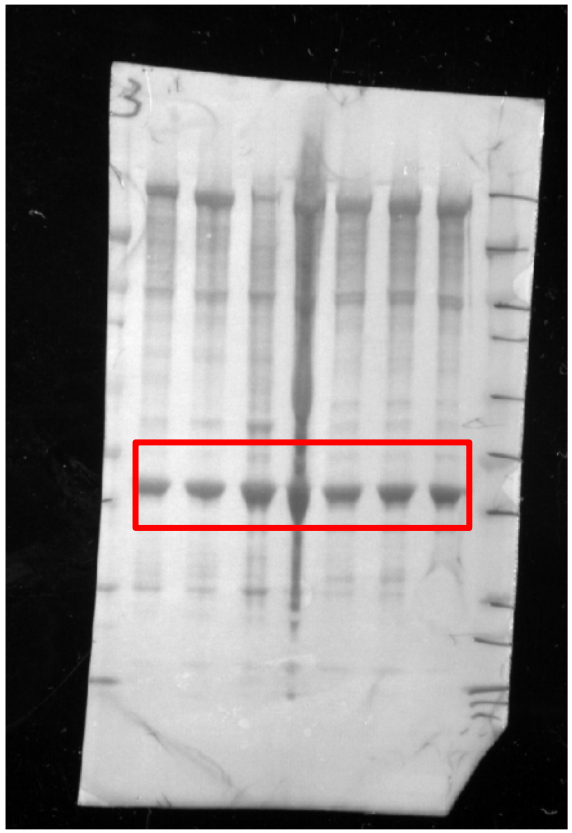


Figure 10B (2/2)

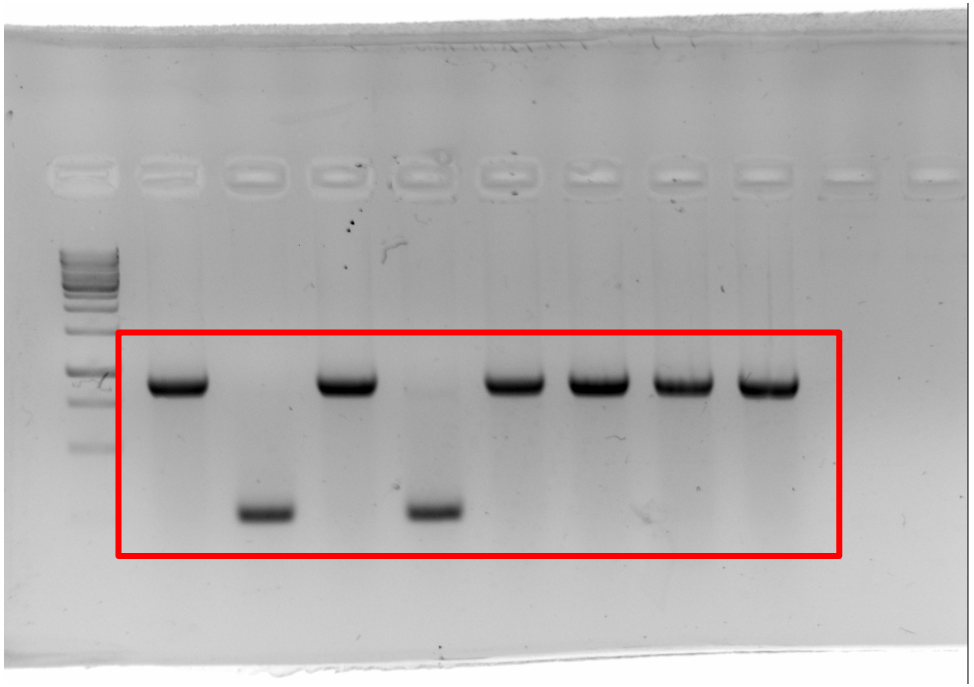
Porin

Ponceau (Actin Band)

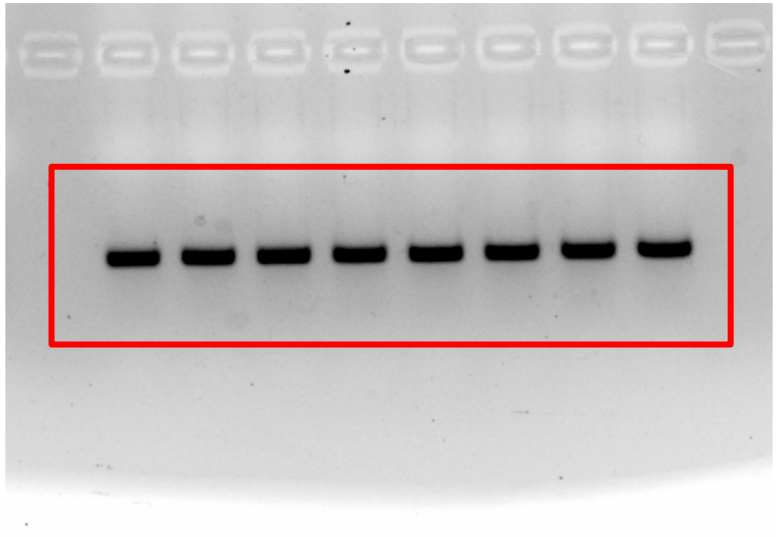


Supplementary Figure 1C (RT-PCR)

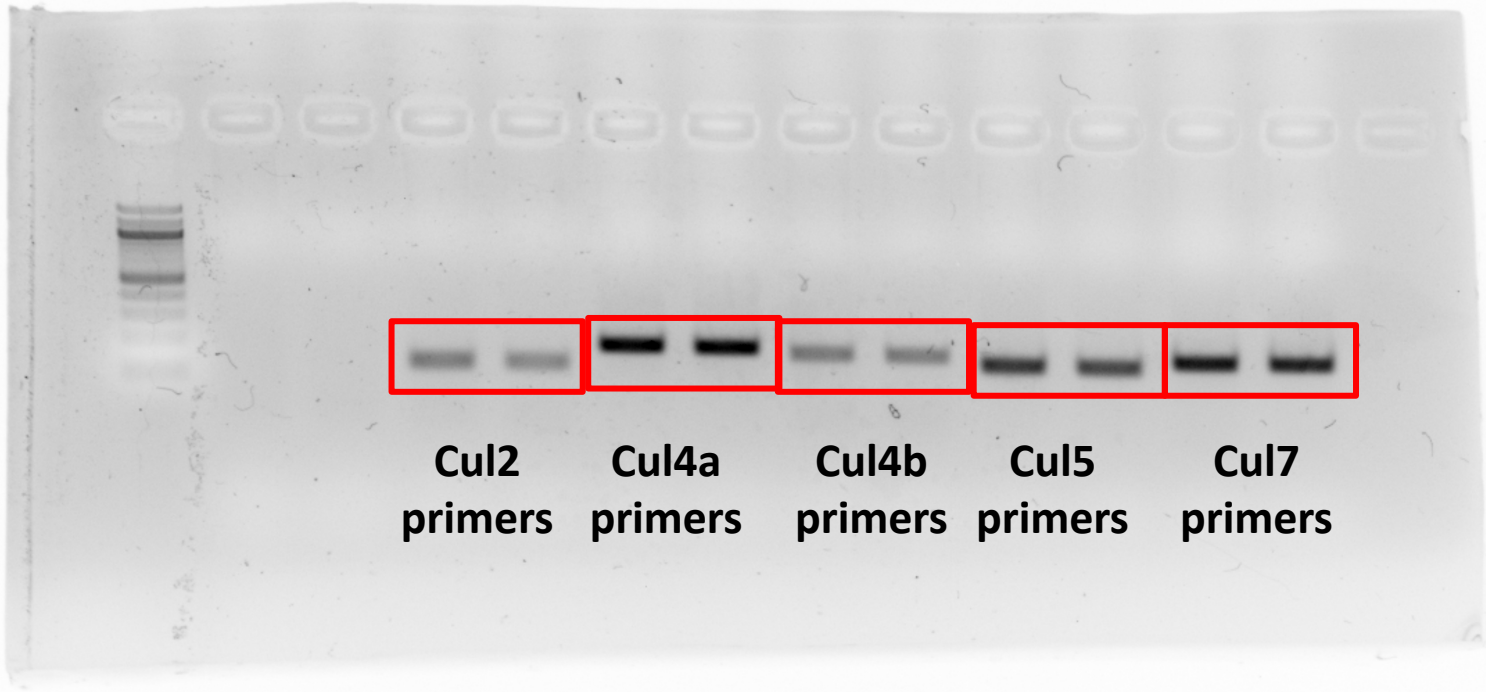
Cul3 primers

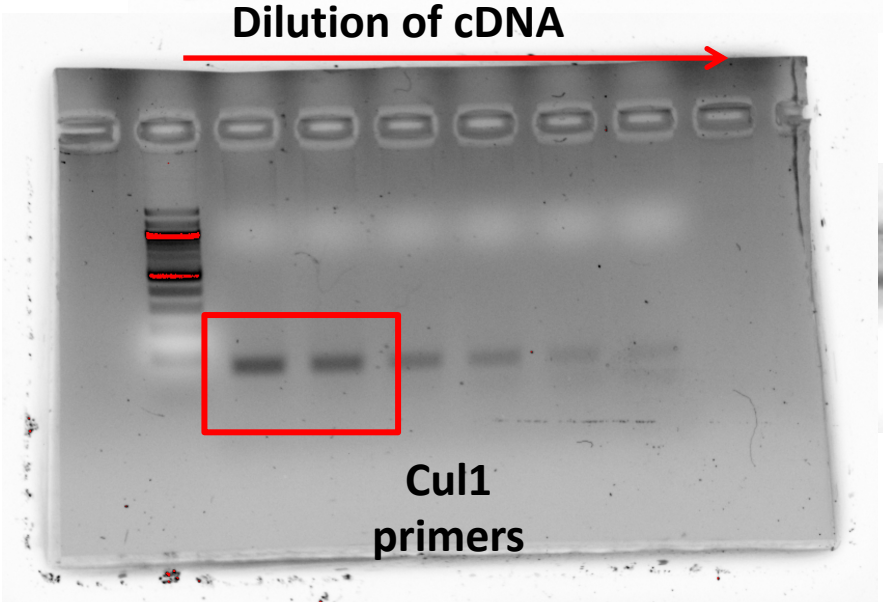


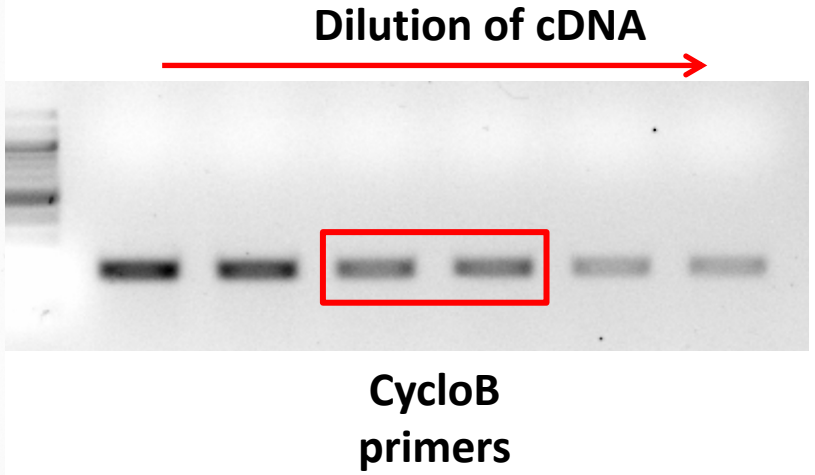
B-Actin primers



Supplementary Figure 1D (RT-PCR)



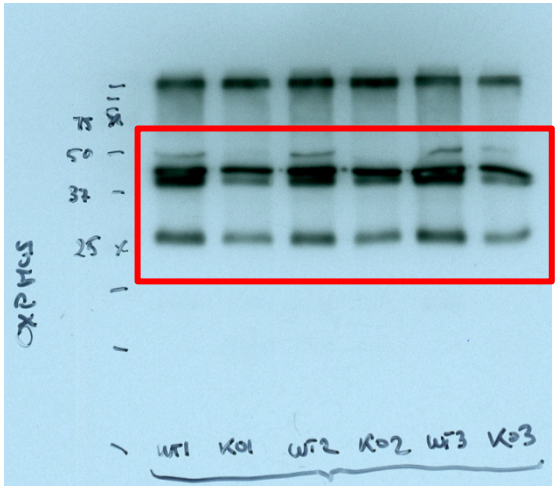




Supplementary Figure 5C

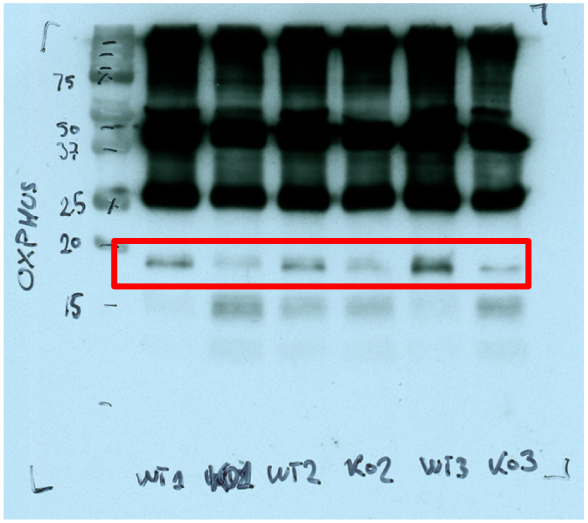
OxPhos:

ATP5A
QCR2
Cox1
SDHB



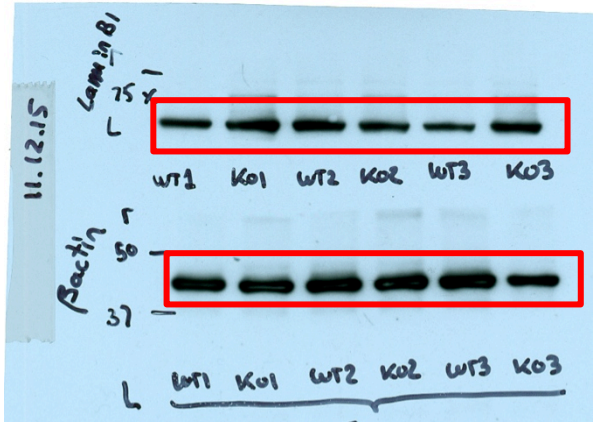
OxPhos:

Nduf8B



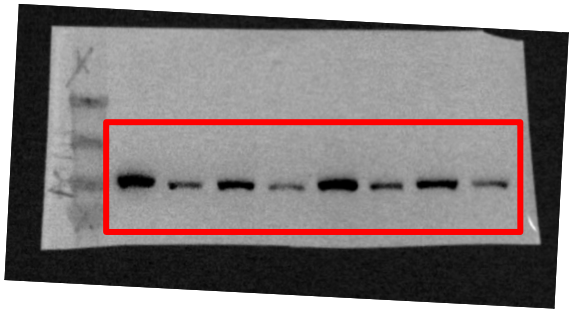
LaminB1

Beta-Actin

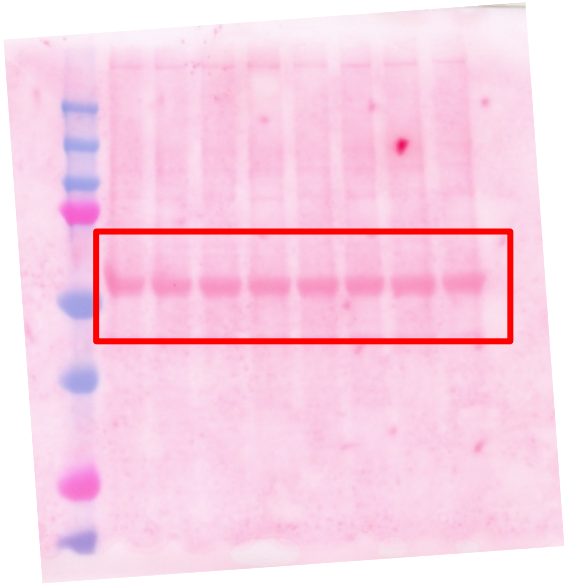


Supplementary Figure 6A

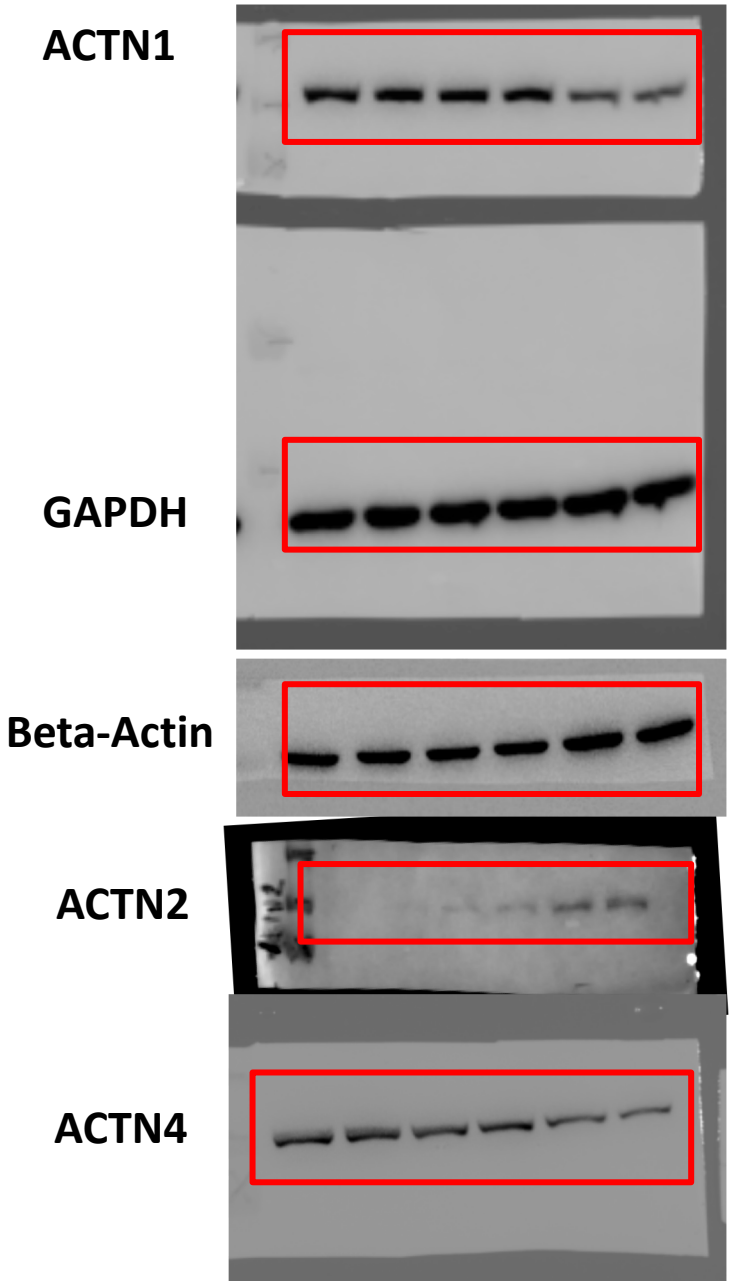
ACTN1



Original Ponceau



Supplementary Figure 6B



Original ponceau