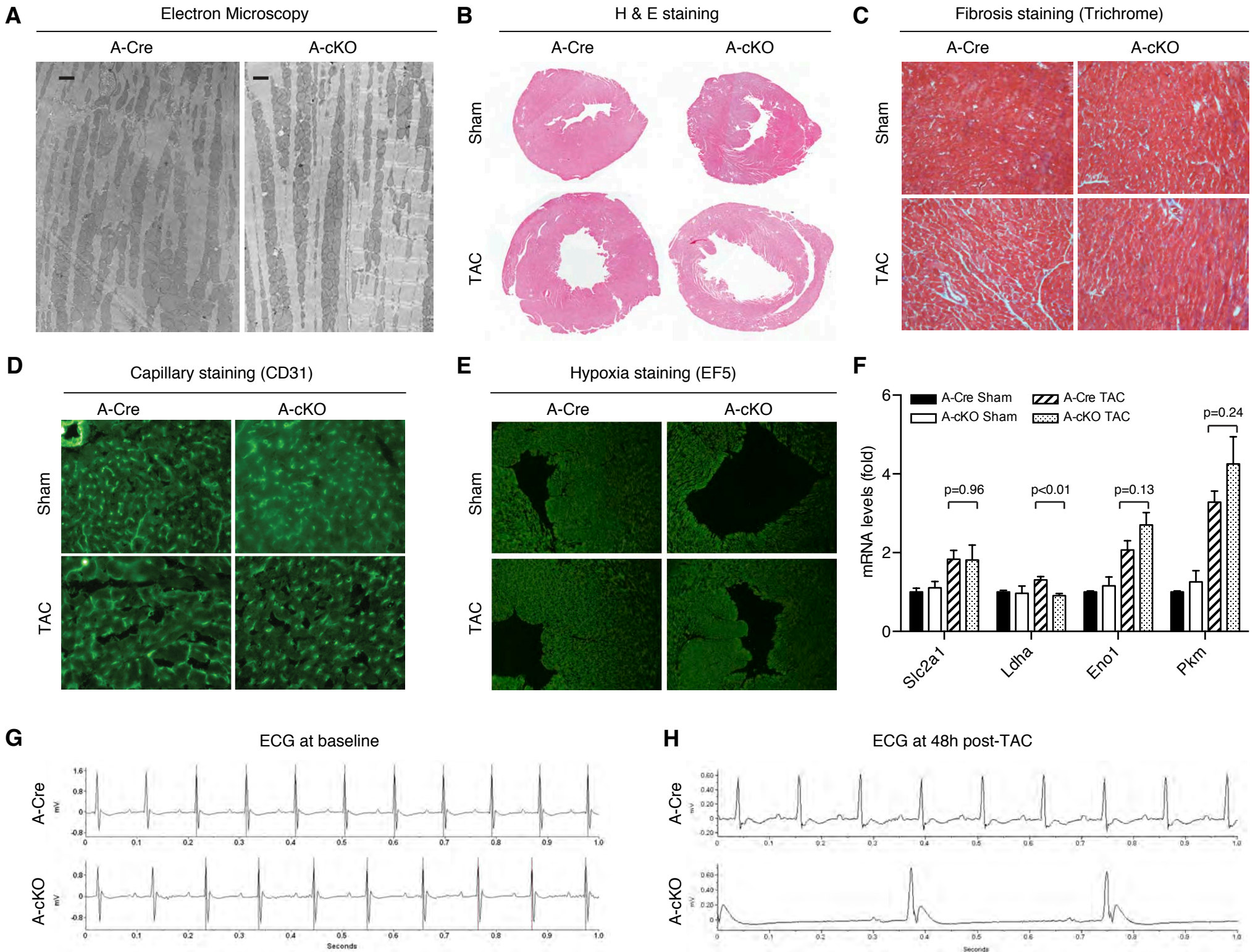


Supplementary Figure 1. Normal structure of A-cKO heart at baseline.

(A) Ultrastructure of myocardium by TEM. Scale bars indicate 1 μm . (B) Hematoxylin & Eosin (H&E) staining of heart tissue. (C) Trichrome staining for fibrosis. (D) Endothelial marker CD31 staining for myocardial capillary density. (E) EF5 staining for hypoxia stress. (F) Expression of hypoxia-inducible genes by qPCR. (G,H) ECG recording by telemetry.



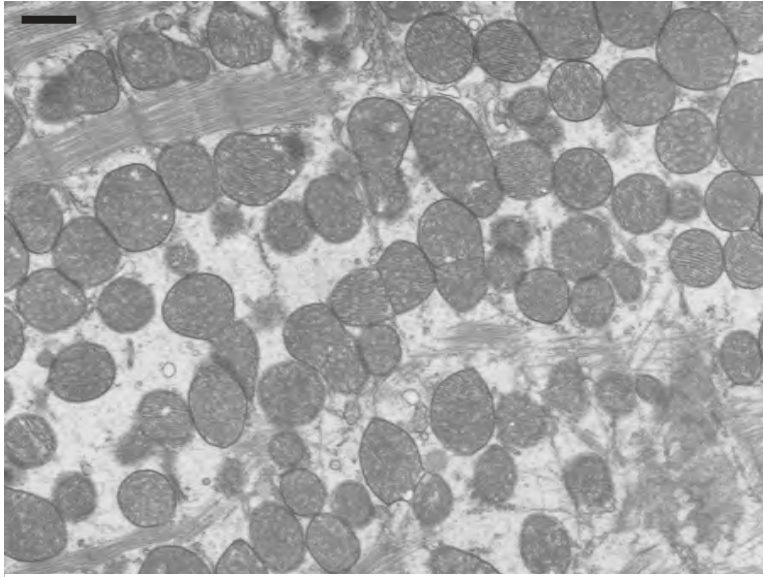
Supplementary Figure 1

Supplementary Figure 2. TAC-induced severe mitochondrial damage in A-cKO heart.

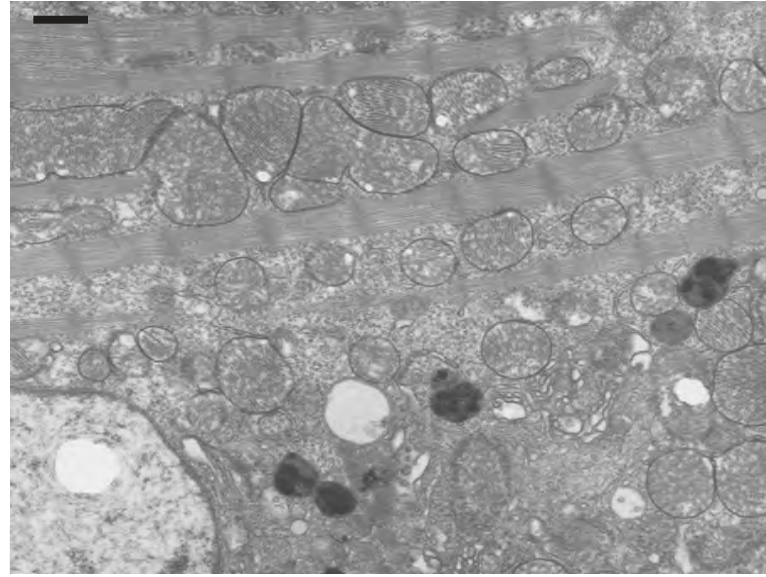
(A) Mitochondrial disarray. (B) Mitochondrial degeneration. (C,D) Altered size and shape of mitochondria. Black arrowheads: fragmented mitochondria. White arrowheads: giant mitochondria. Scale bars indicate 1 μm .

A

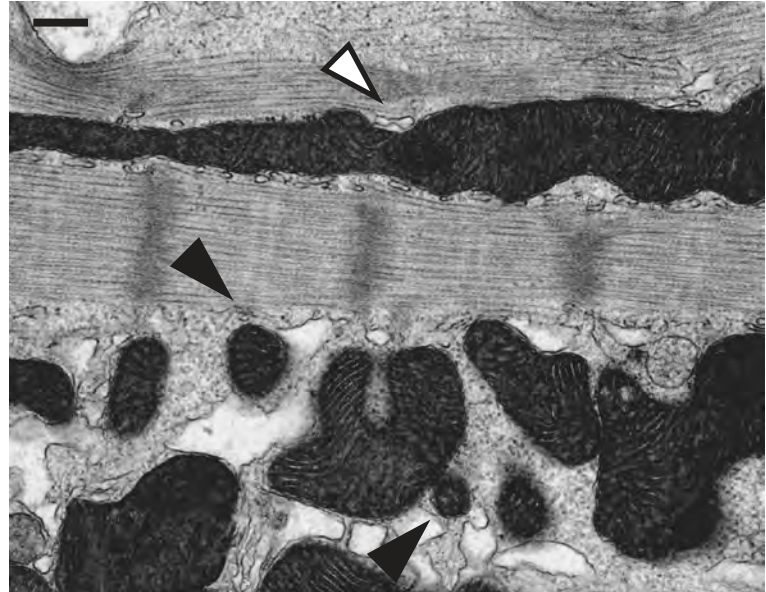
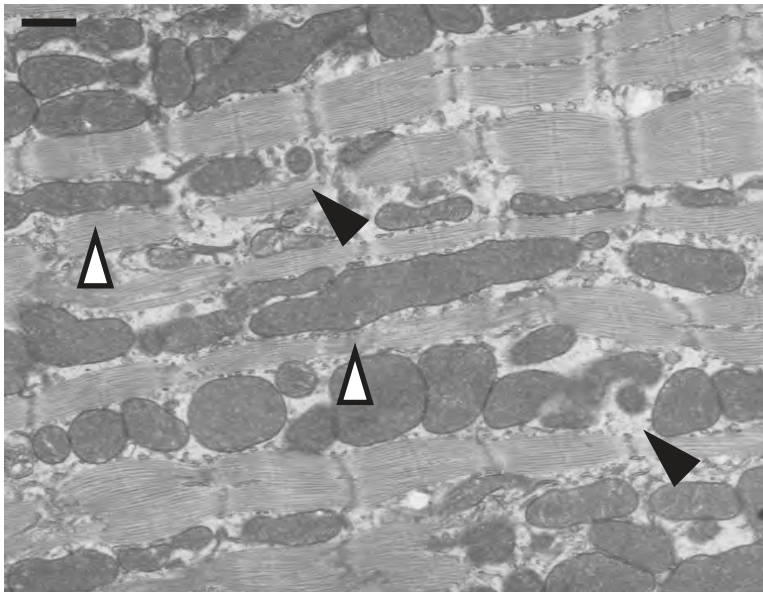
Mitochondrial disarray

**B**

Mitochondrial degeneration

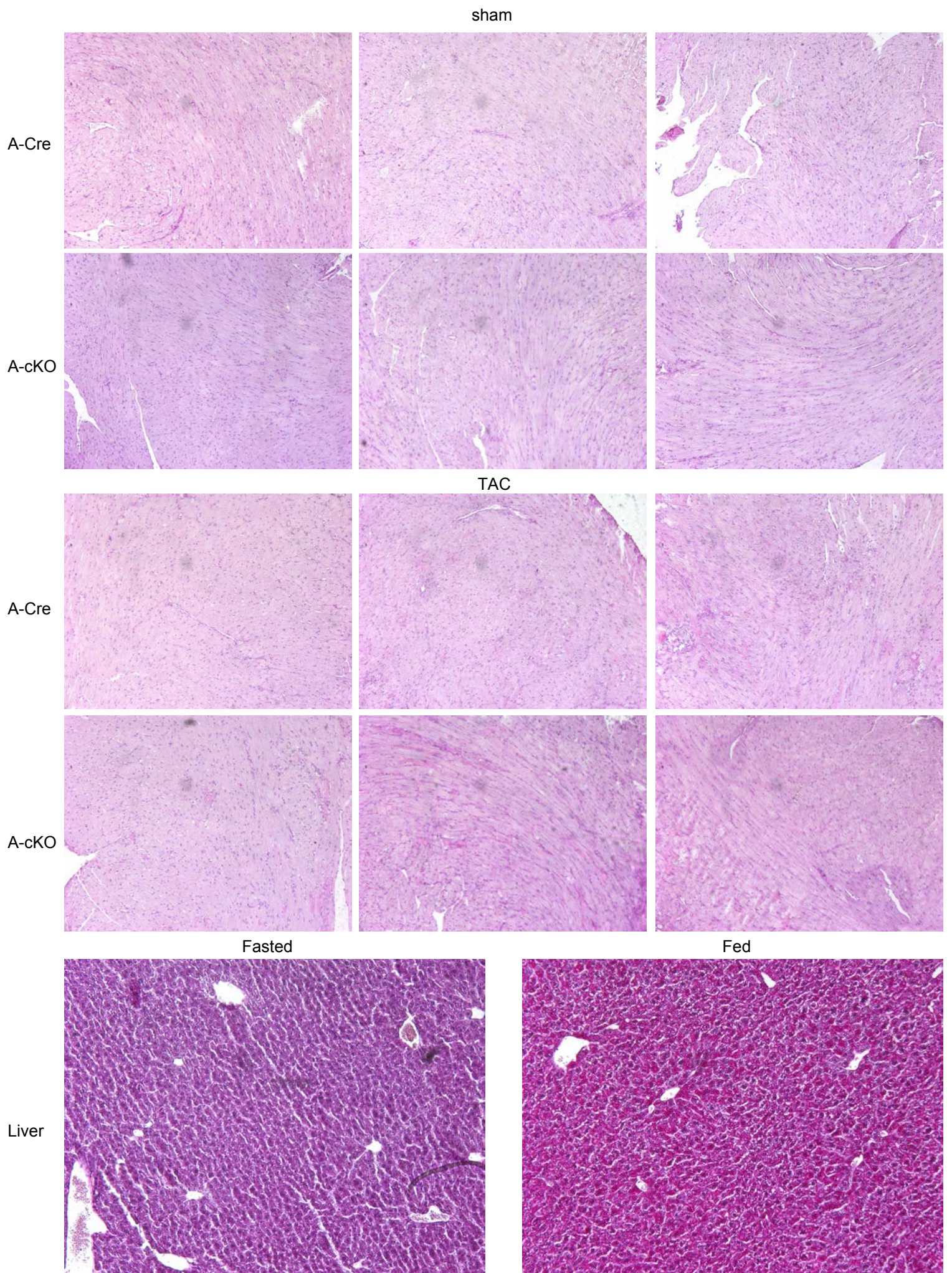
**C**

Mitochondrial fragmentation and formation of giant mitochondria



Supplementary Figure 3. Myocardial glycogen content before and after 3-day of TAC.

Glycogen deposition was assessed by PAS staining. n=3 in each group. Liver sections from normally fed (Fed) and 24-hour fasted (Fasted) mice were included as controls. Glycogen deposits stained in red.

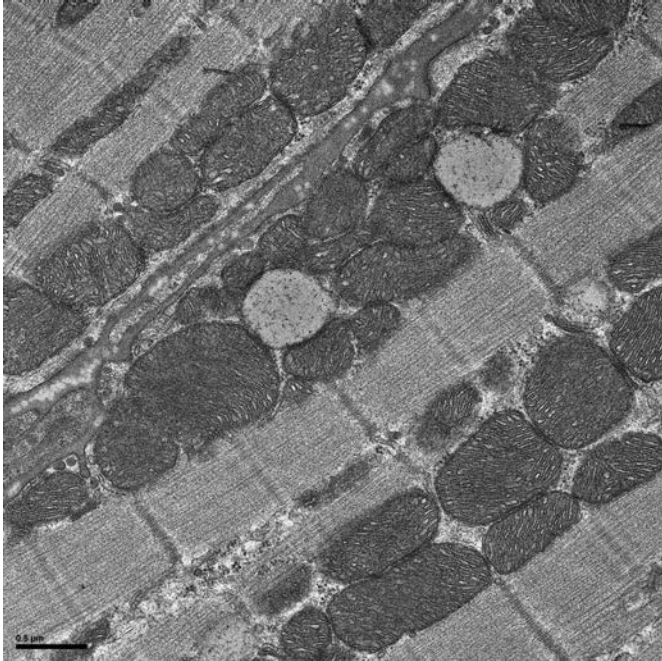


Supplementary Figure 3

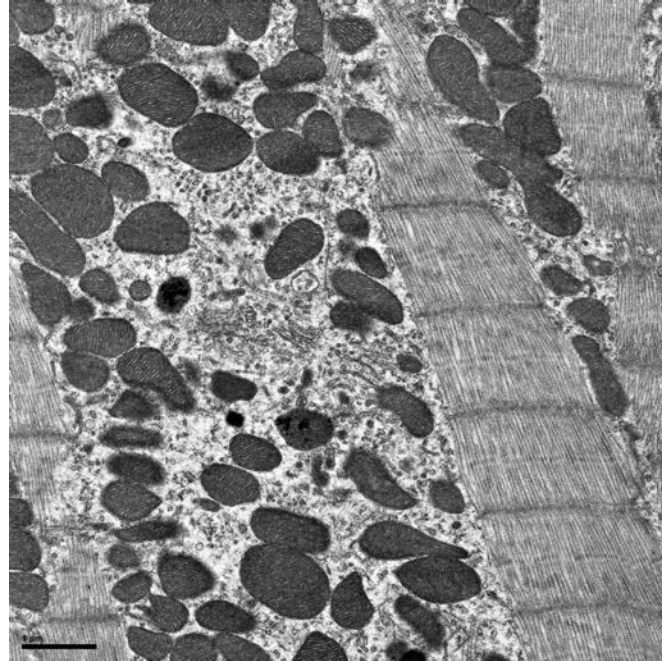
Supplementary Figure 4. Mitochondrial dysfunction in aged KLF4-deficient hearts.

Mice were 9-month old. Scale bars indicate 0.5 μm . Representative images from 3 animals in each group were shown.

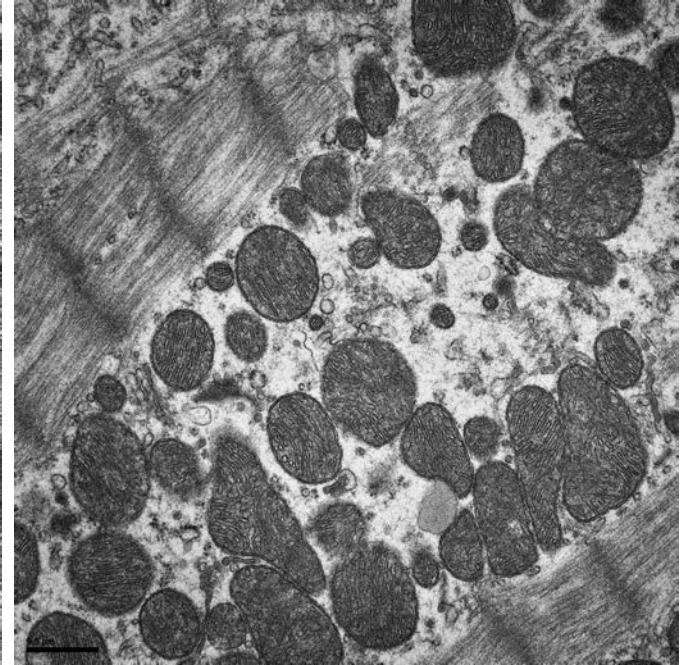
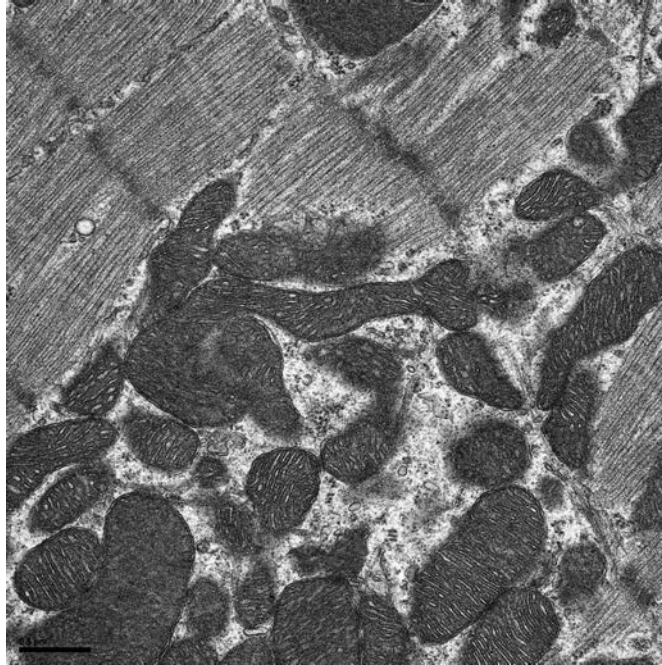
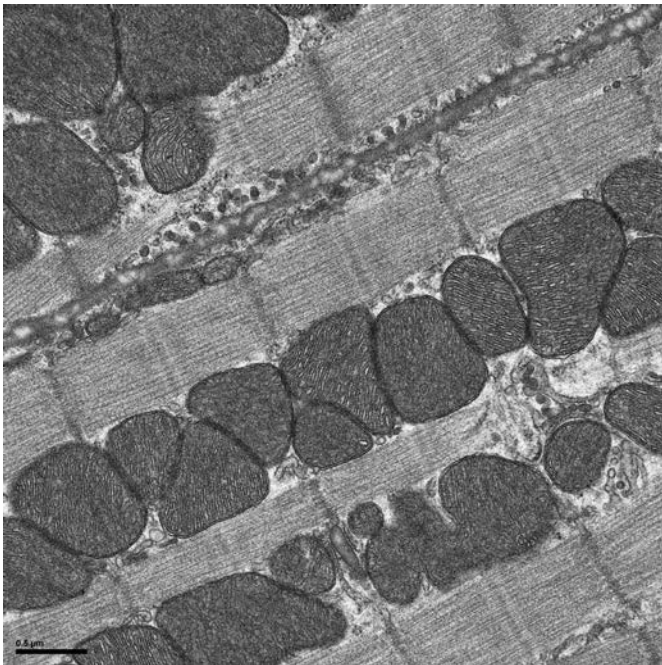
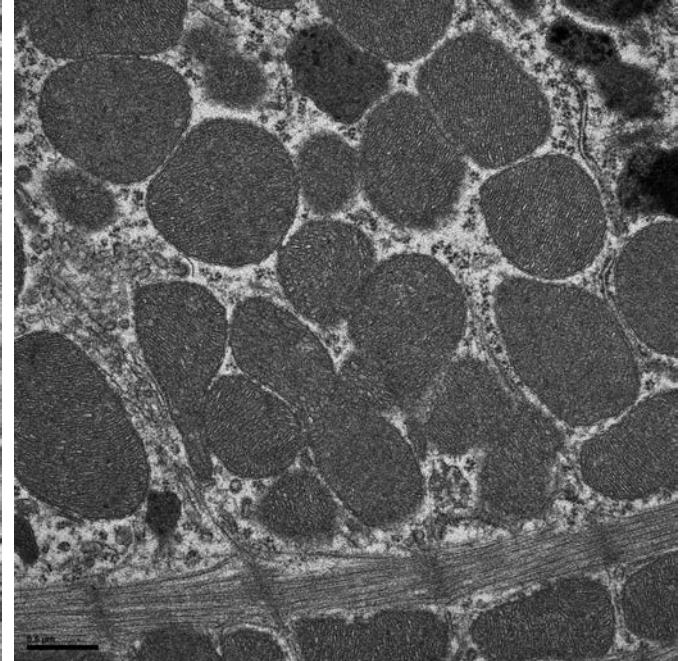
A-Cre



A-cKO



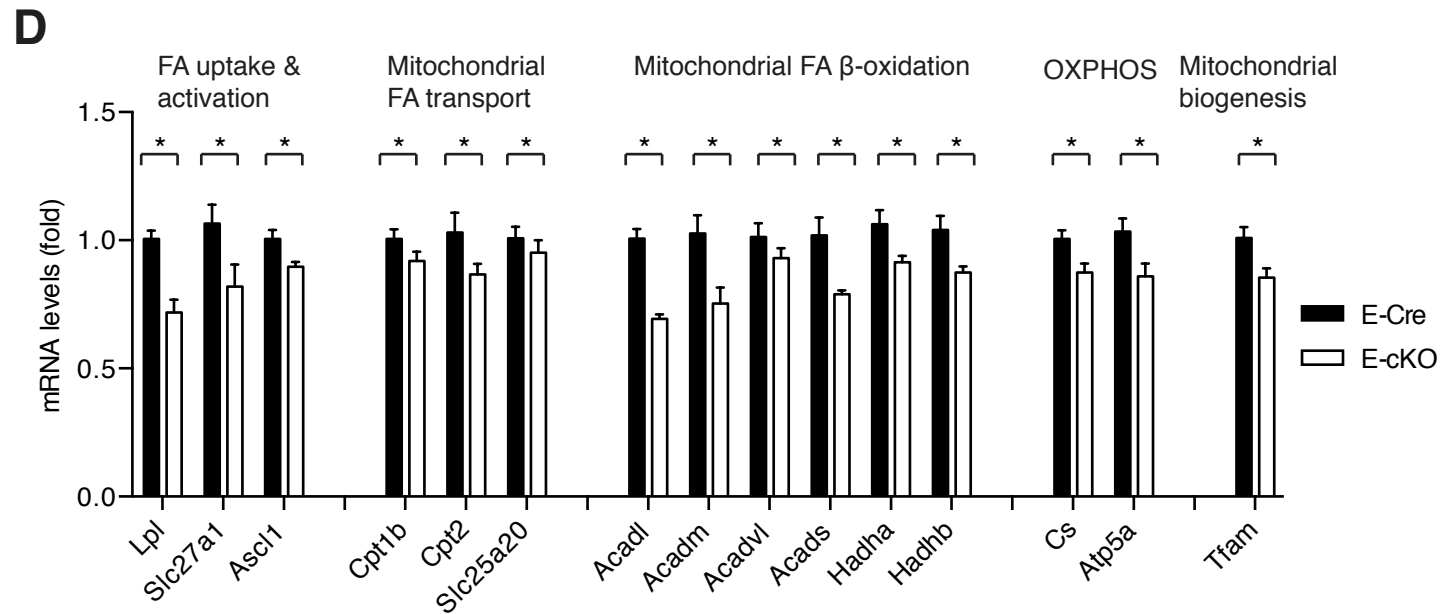
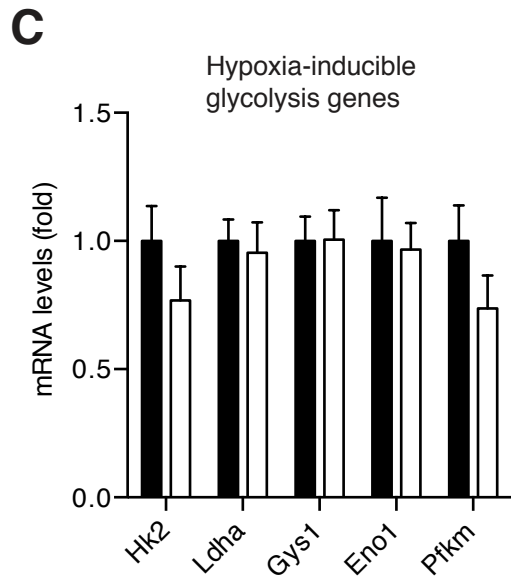
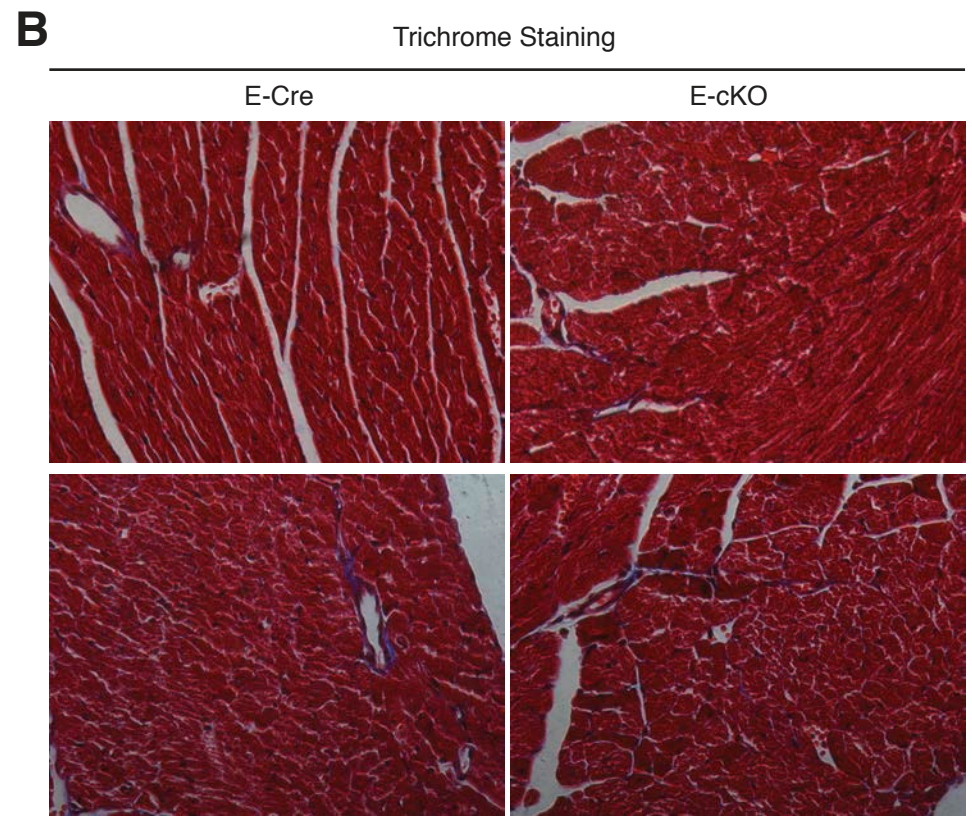
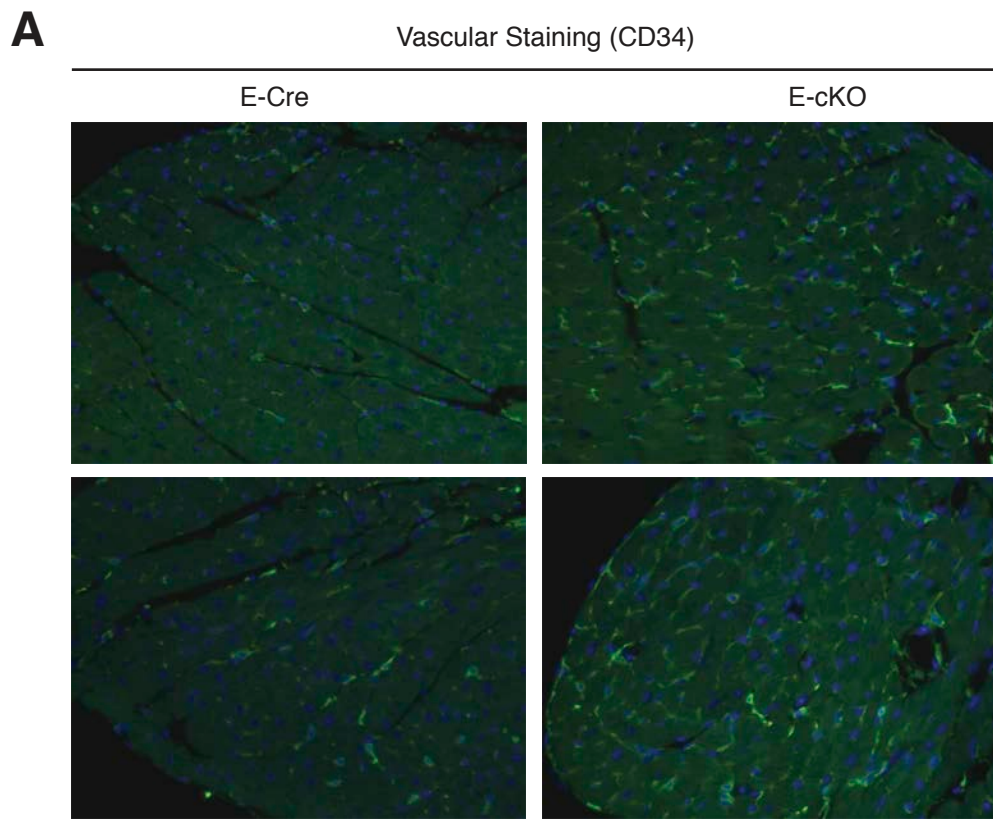
A-cKO



Supplementary Figure 5. Normal vascular development and gross structure of E-cKO heart.

(A) Capillary density revealed by CD34 staining. (B) Trichrome staining to reveal fibrosis.

Representative images from 3 pairs of animals were shown. (C) Expression of hypoxia-inducible glycolysis genes. (D) Expression of metabolic and mitochondrial genes. Genes expression was determined in the heart at 2-week of age. n=10 in each group. *p<0.05.

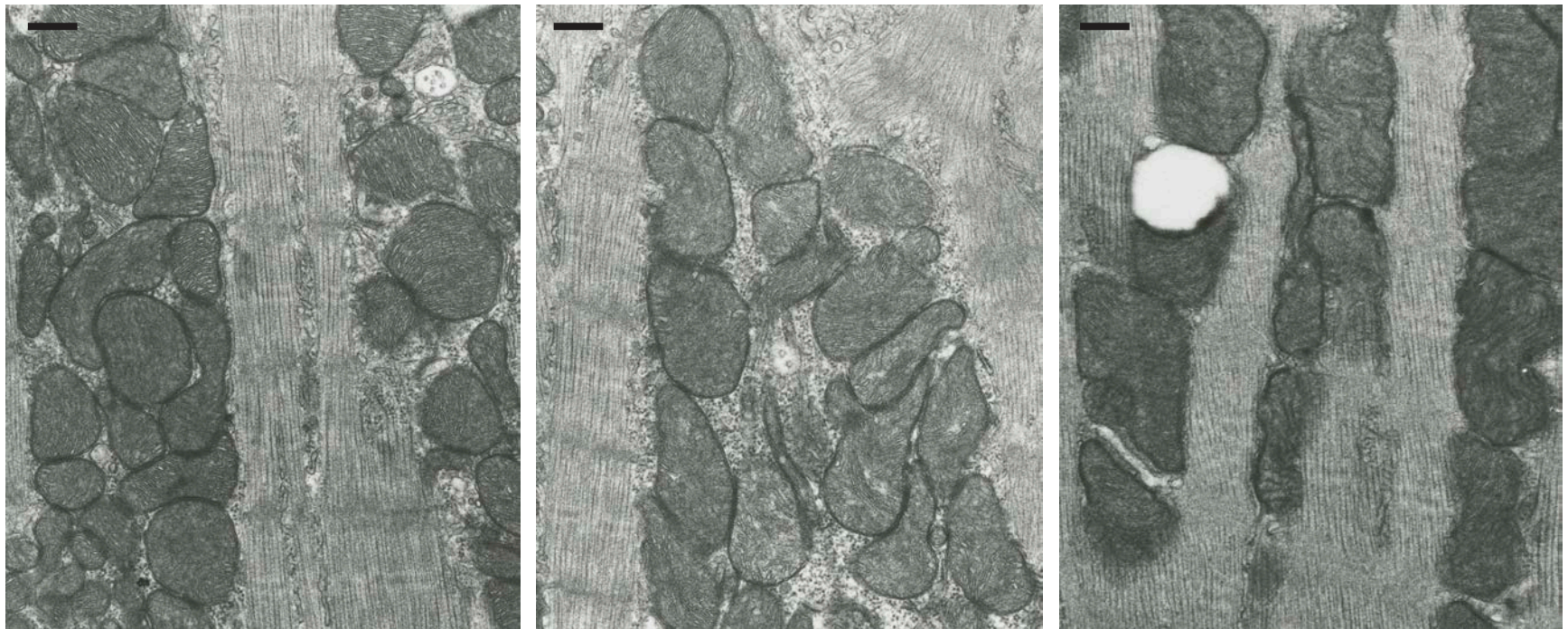


Supplementary Figure 6. Mitochondrial abnormality in E-cKO hearts at 2-week after birth.

(A) Normal ultrastructure of E-Cre hearts. (B) Abnormality of myocardial ultrastructure in E-cKO hearts showing mitochondrial disarray, degeneration, and altered size and shape of mitochondria. Red arrowheads: degenerated mitochondria. Black arrowheads: fragmented mitochondria. White arrowheads: giant mitochondria. Scale bars indicate 500 nm.

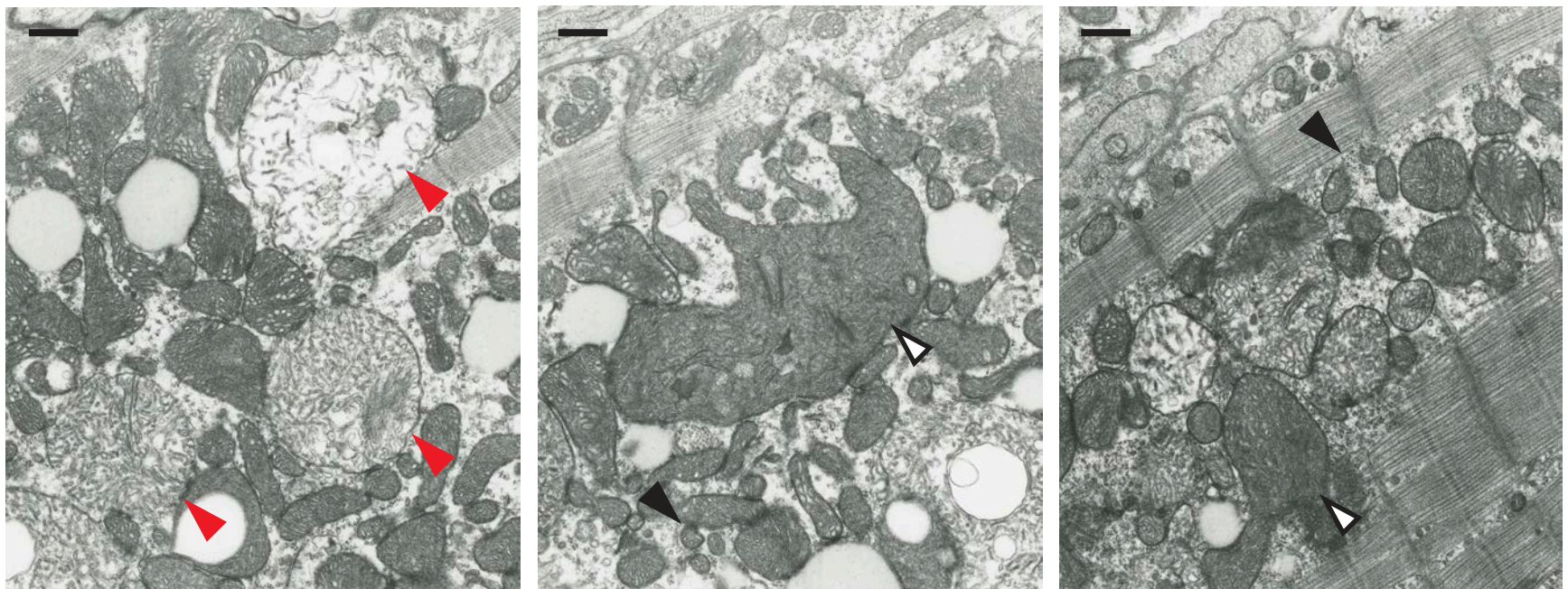
A

E-Cre



B

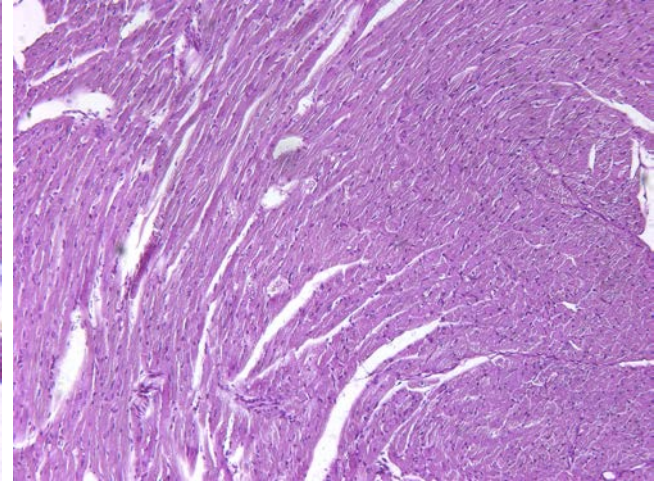
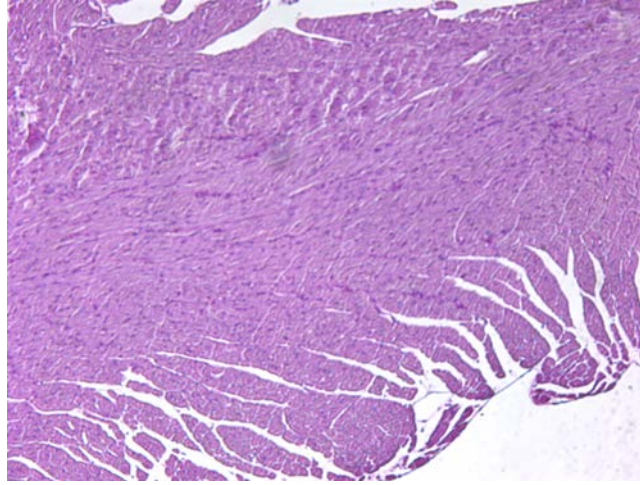
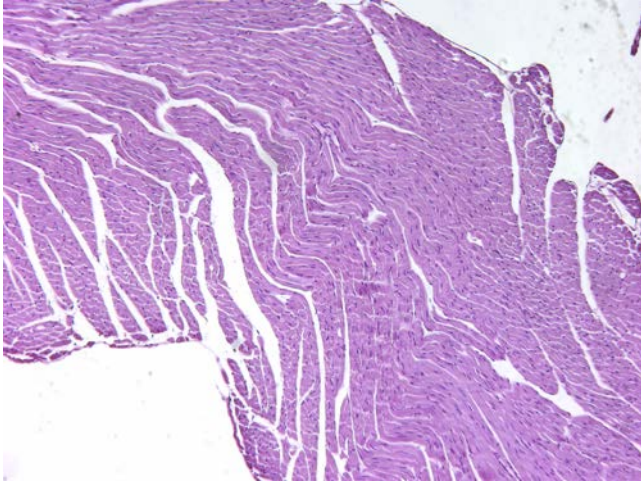
E-cKO



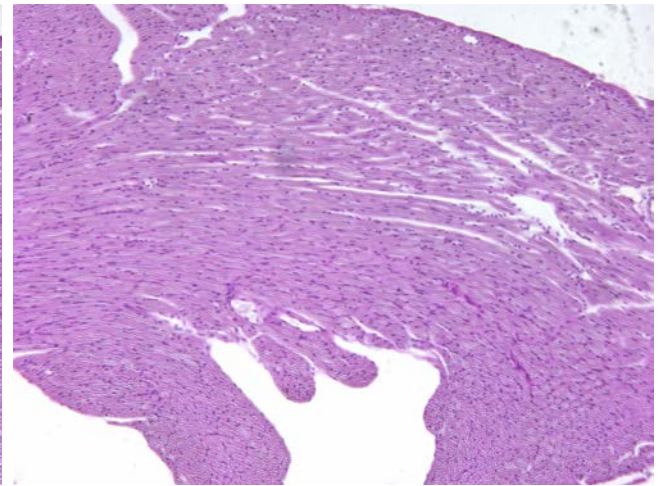
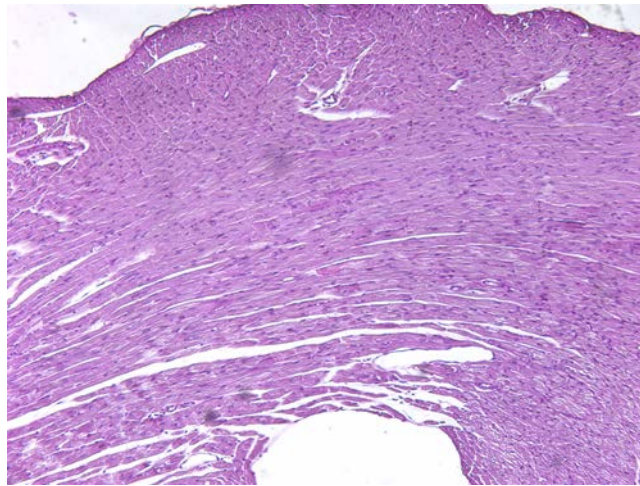
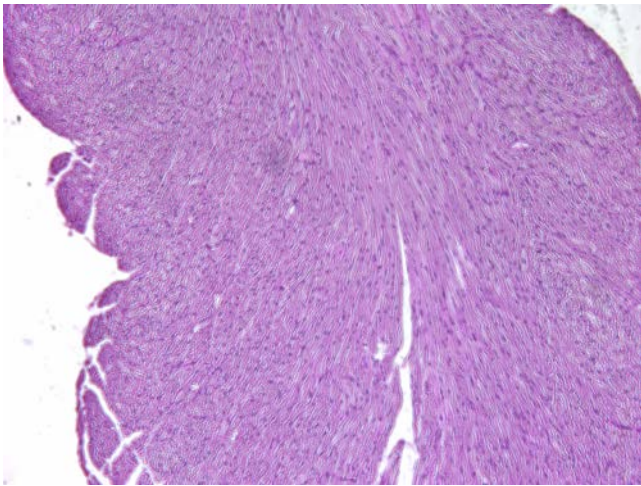
Supplementary Figure 7. Myocardial glycogen content in 3-week-old mice.

Glycogen deposition was assessed by PAS staining. n=3 in each group. Glycogen deposits stained in red.

E-Cre

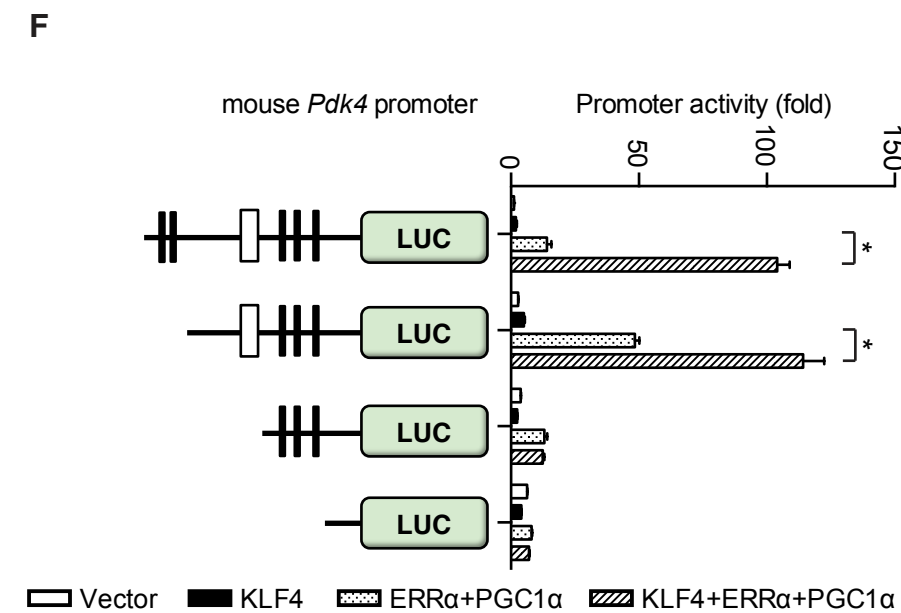
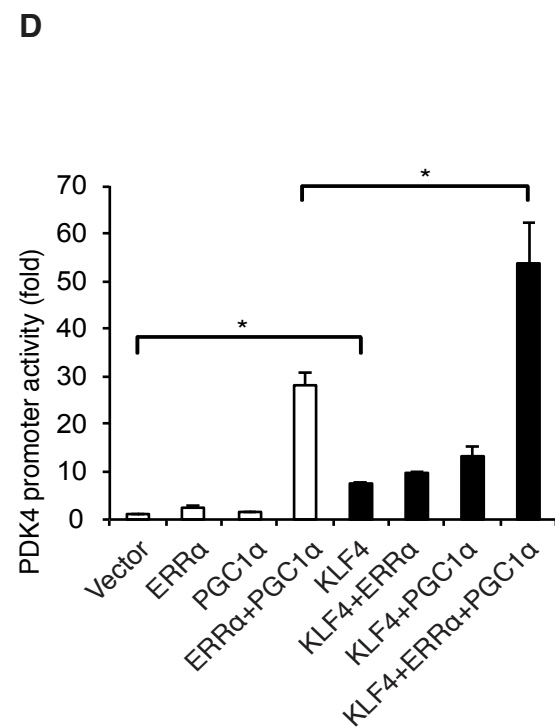
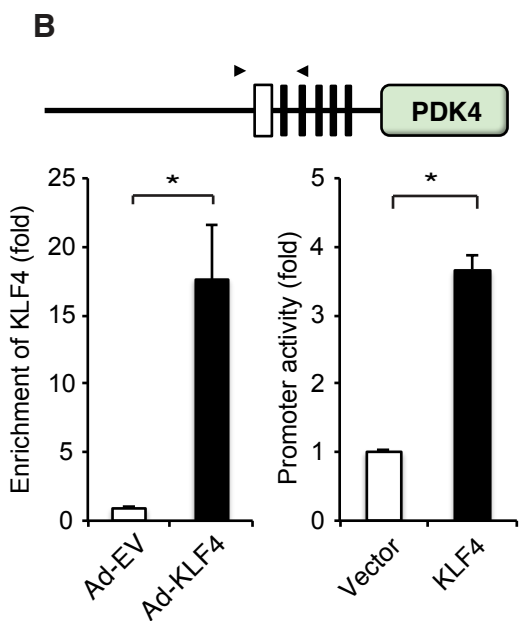
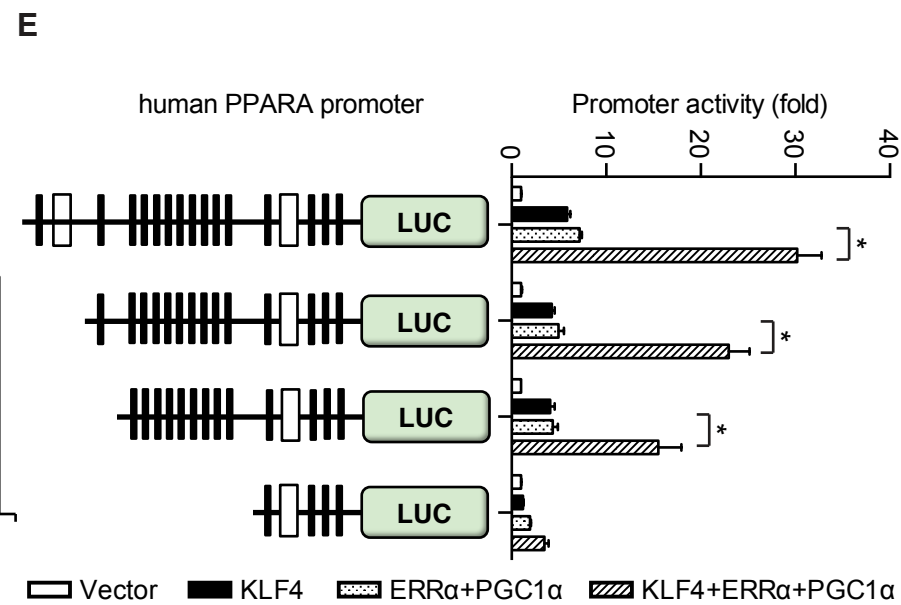
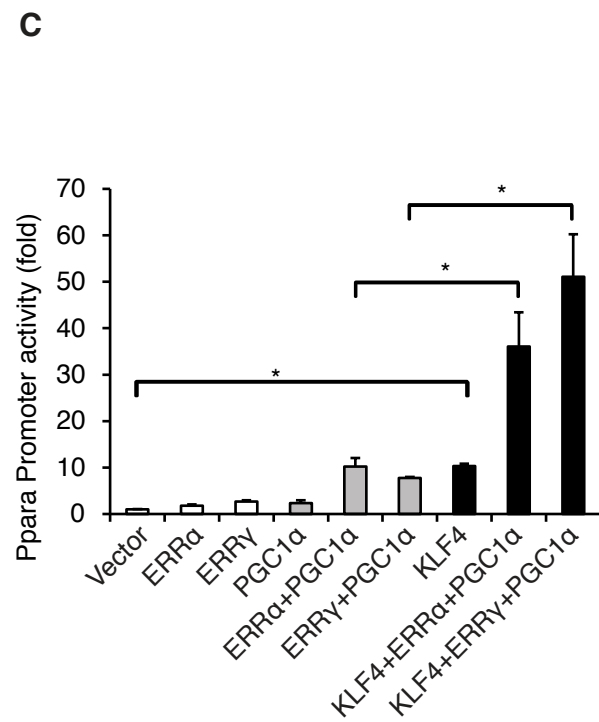
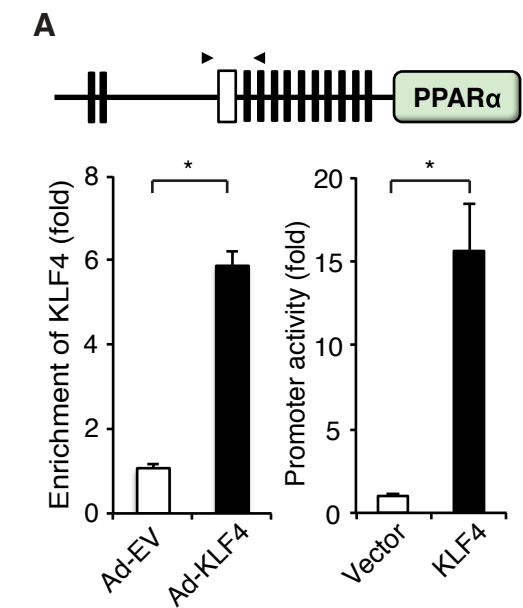


E-cKO



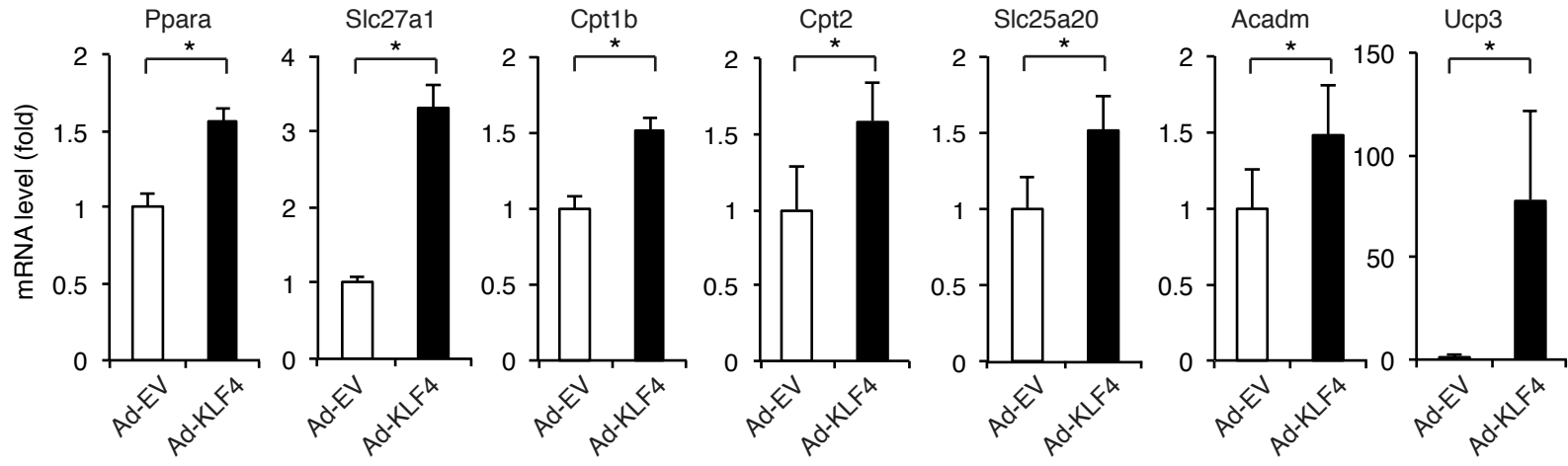
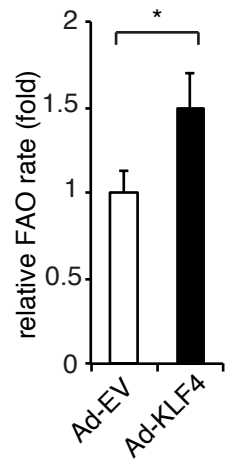
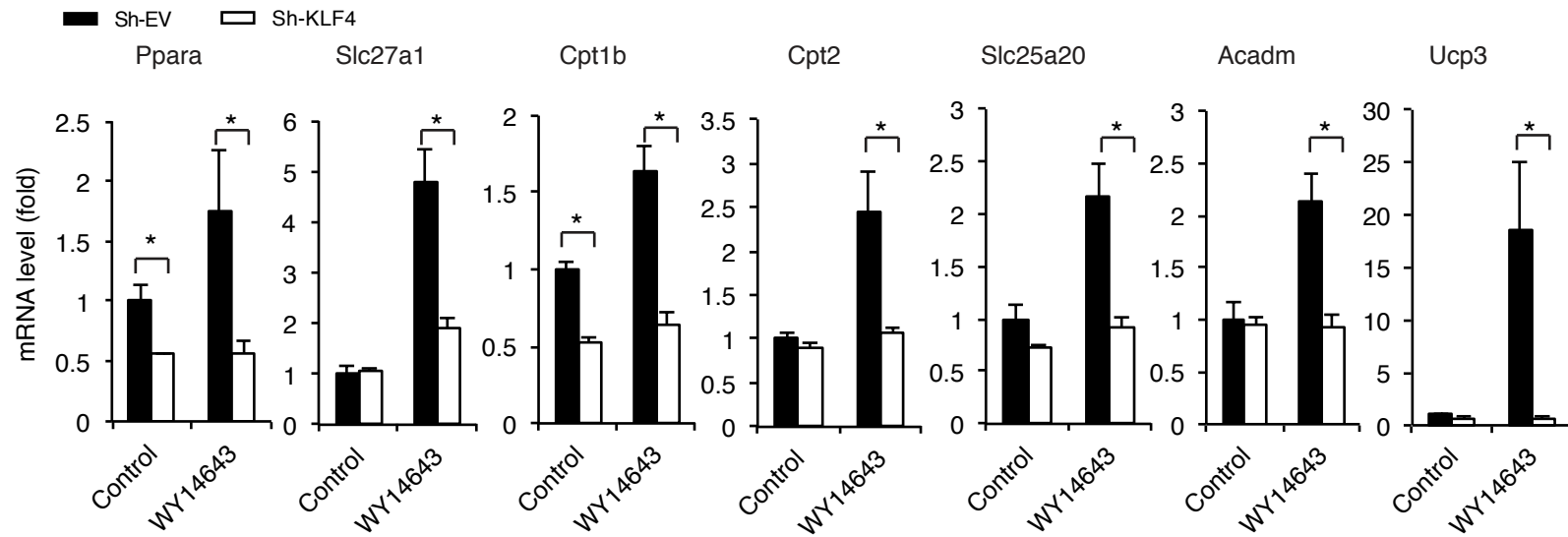
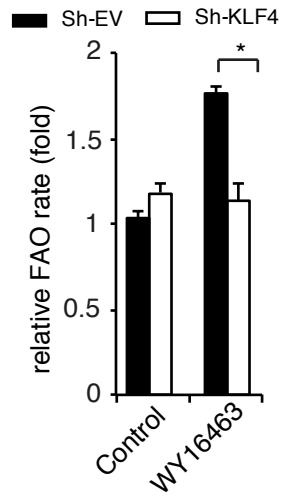
Supplementary Figure 8. Interaction and cooperativity between KLF4 and the ERR/PGC-1 module.

(A, D) Recruitment of KLF4 onto endogenous *Ppara* (A) and *Pdk4* (D) promoters and activation of the promoter reporter. Solid bars indicate KRE and open bar indicates NRRE. Arrow heads indicate PCR regions in CHIP assays. (B, E) Synergistic cooperativity between KLF4 and the ERR/PGC-1 complex on target promoters. (C, F) Promoter truncation analysis. n=3. *p<0.05.



Supplementary Figure 9. KLF4 positively regulates and is requisite for PPAR α signaling.

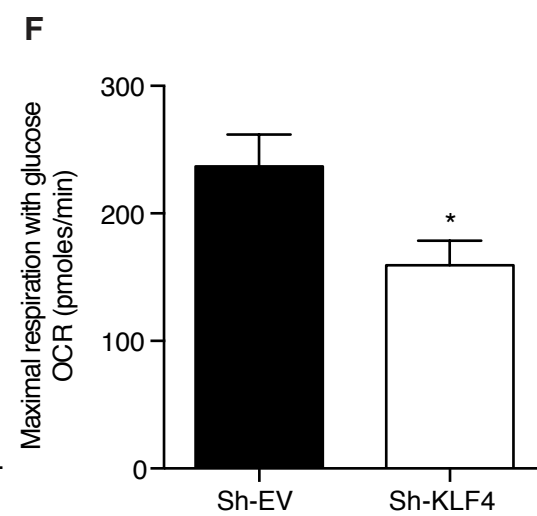
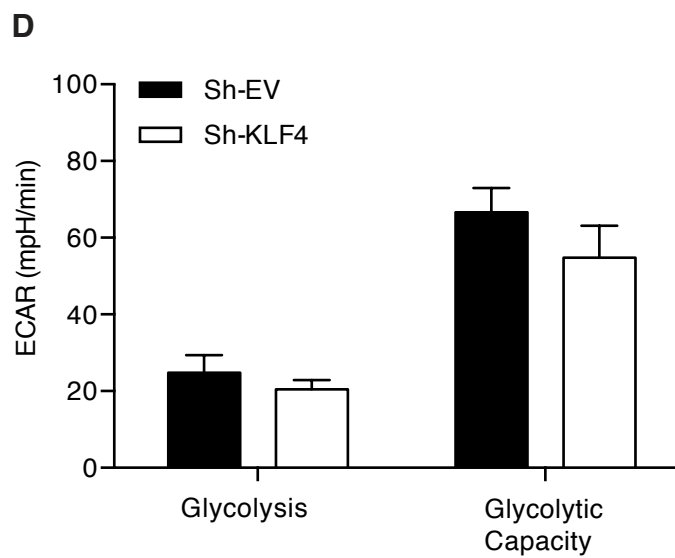
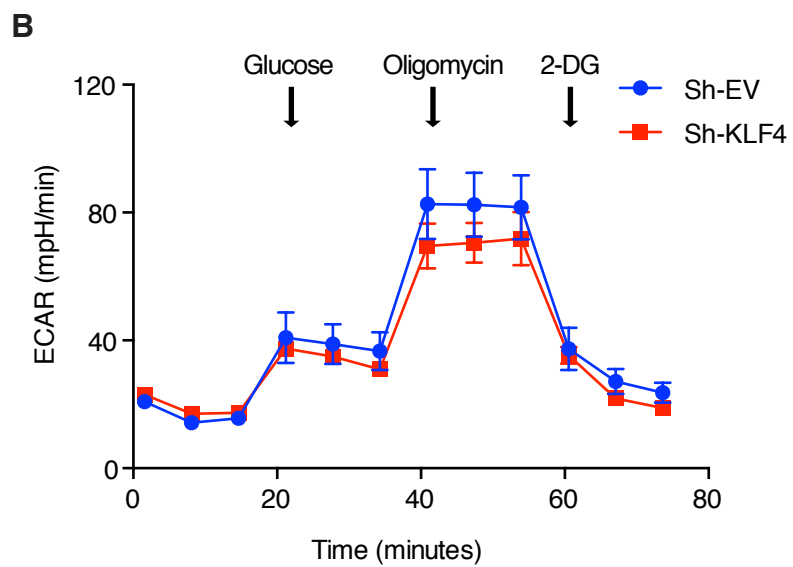
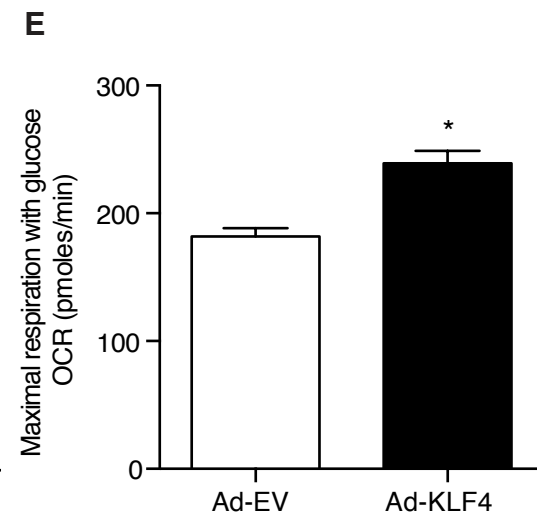
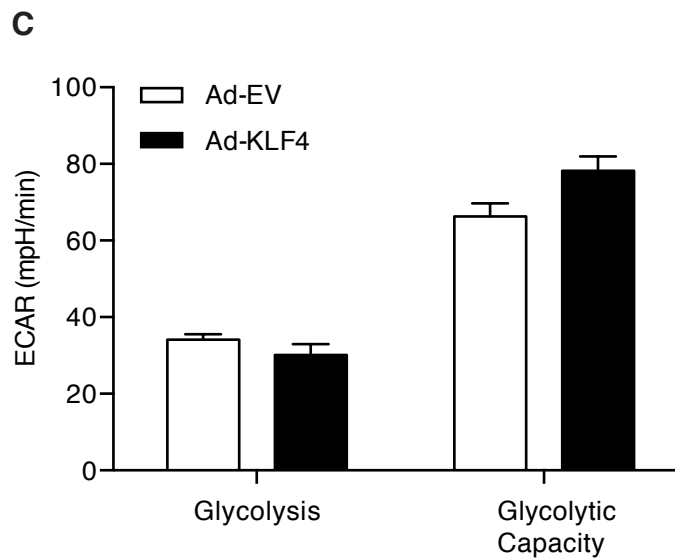
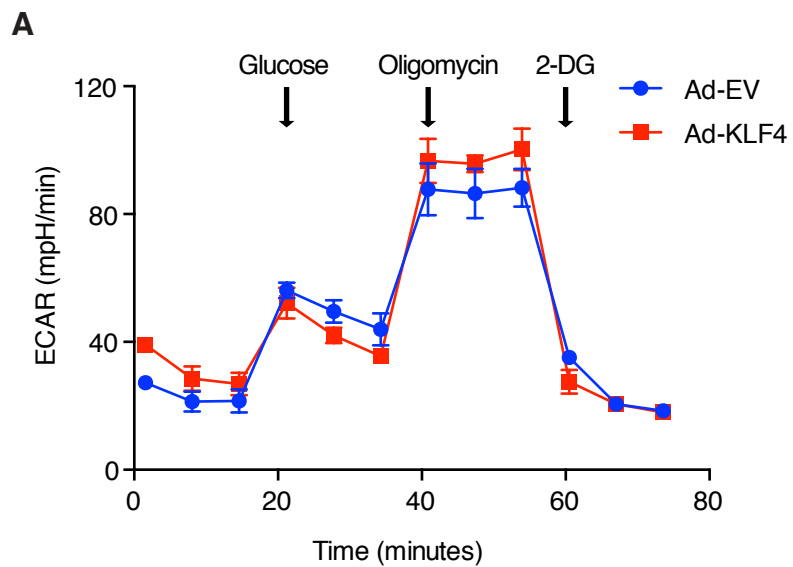
(A, B) Adenoviral overexpression of KLF4 in NRVM induced PPAR α target genes (A) and enhanced FAO (B). (C, D) Adenoviral knockdown of KLF4 in NRVM impaired PPAR α ligand WY14643-induced target genes expression and induction of FAO. NRVM were infected with adenovirus for 48 h before treatment with WY14643 for 16 h. n=3 in each group. *p<0.05.

A**B****C****D**

Supplementary Figure 10. Effects of KLF4 on cardiac glucose metabolism.

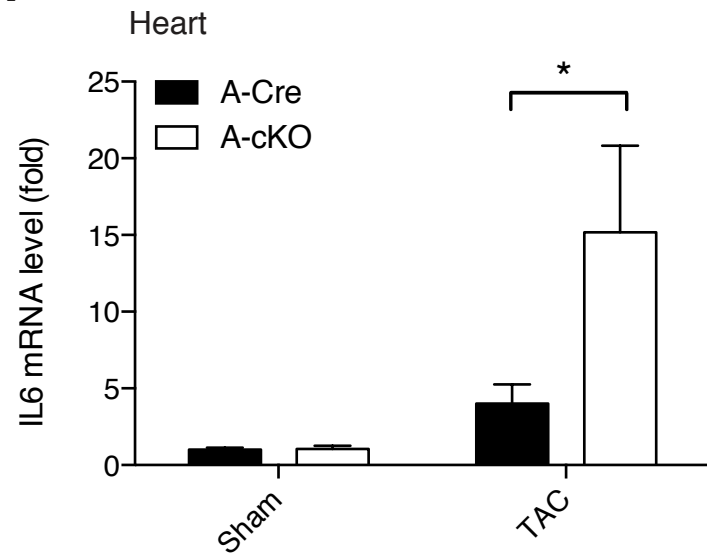
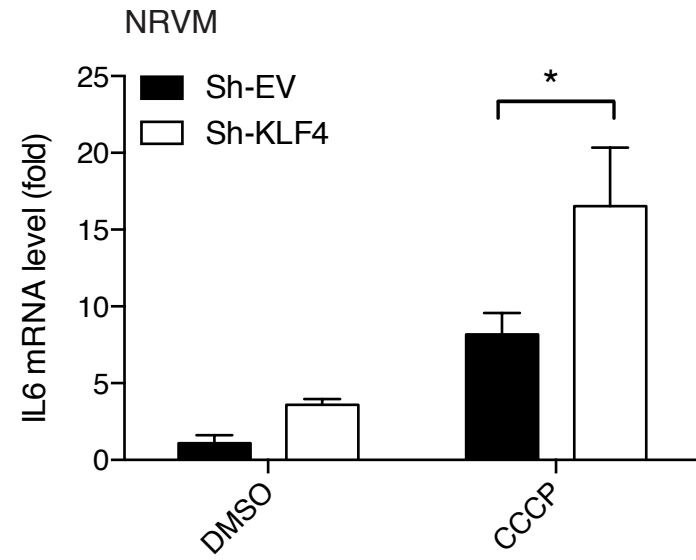
(A, B) Glycolysis assays using the Seahorse glycolysis stress test in NRVM with acute overexpression (A) or knockdown (B) of KLF4. (C, D) Glycolysis rate shown as extracellular acidification rate (ECAR). Basal glycolysis rate was calculated as $\Delta(\text{ECAR}_{\text{glucose}} - \text{ECAR}_{\text{basal}})$. Glycolysis capacity was calculated as $\Delta(\text{ECAR}_{\text{Oligomycin}} - \text{ECAR}_{\text{basal}})$. (E, F) Maximal glucose oxidation rate was calculated from FCCP-induced oxygen consumption rate (OCR) with glucose (25 mmol/L) as the sole substrate using the Seahorse cell mito stress test. n=3 in each group.

*p<0.05.



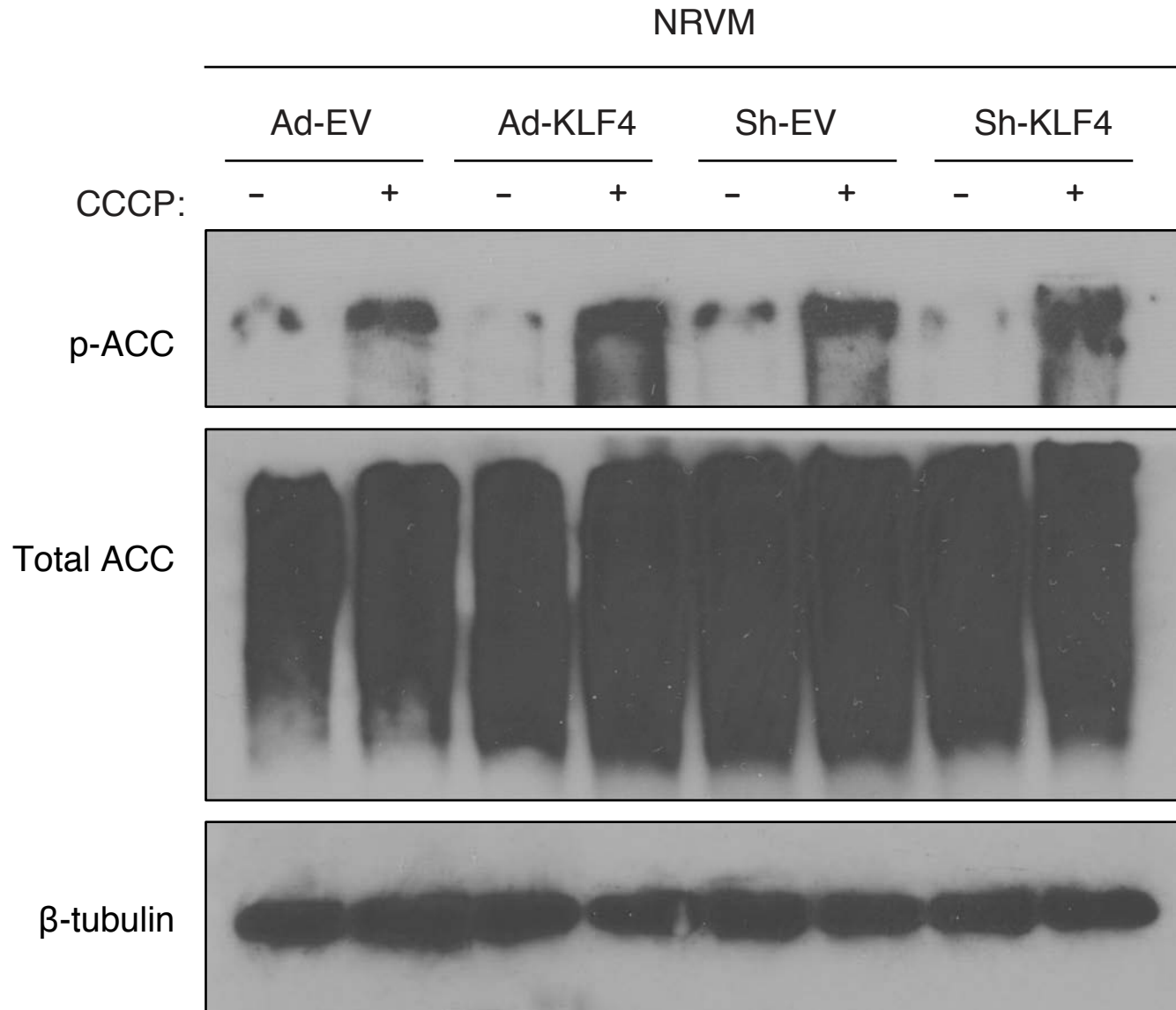
Supplementary Figure 11. Impaired mitophagy was associated with inflammation.

(A) IL6 mRNA expression in KLF4-deficient myocardium. (B) IL6 mRNA expression in KLF4-deficient cardiomyocytes. n=3 in each group. *p<0.05.

A**B**

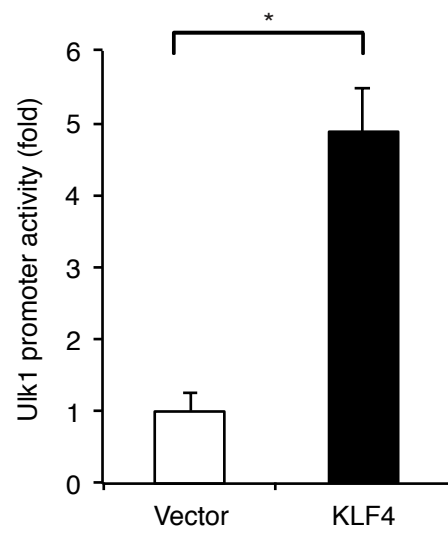
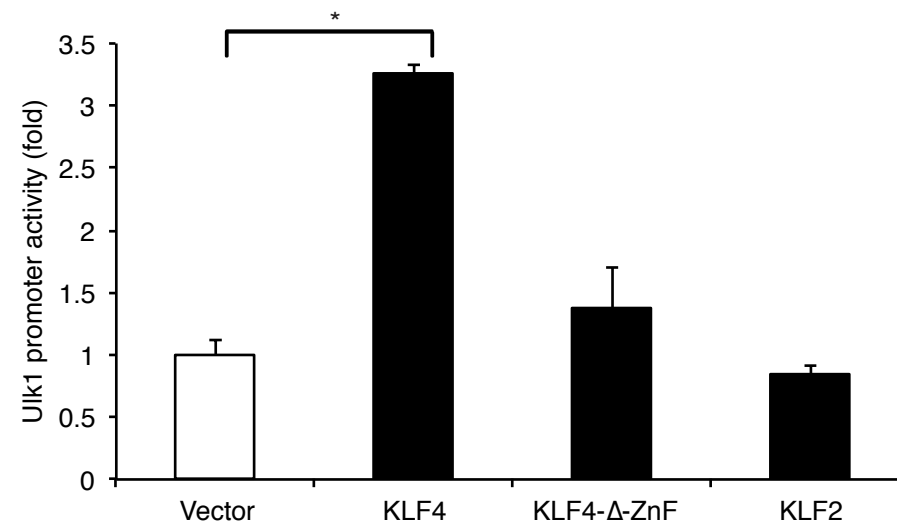
Supplementary Figure 12. KLF4 does not affect AMPK-mediated phosphorylation of ACC in NRVM.

Western blot for total protein of Acetyl-CoA Carboxylase (ACC) and its Ser79-phosphorylated form in NRVM. ACC is a confirmed direct target of AMPK.



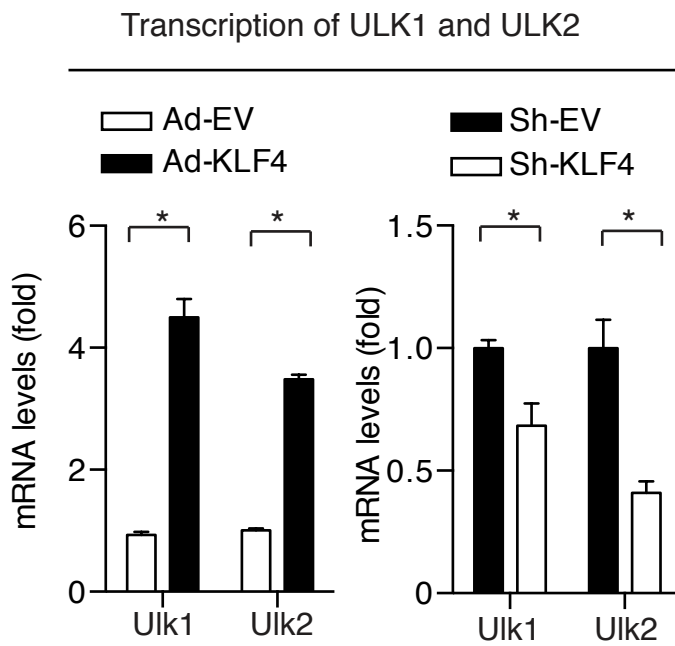
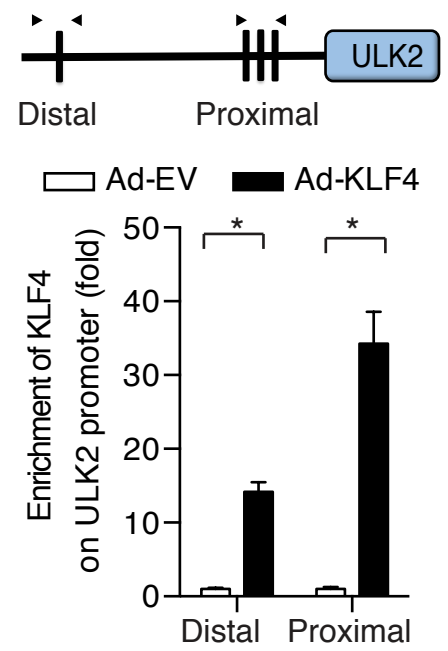
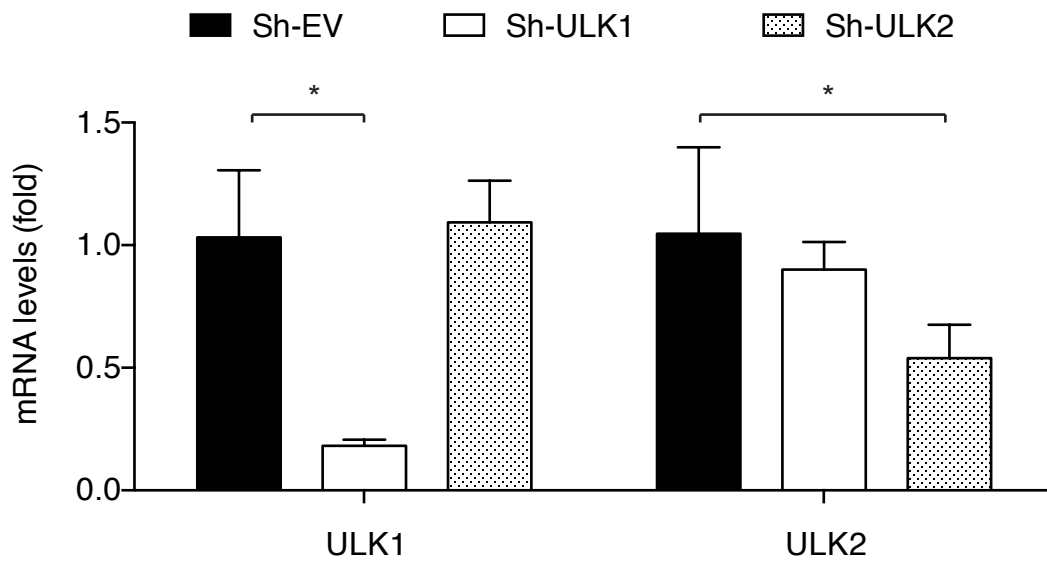
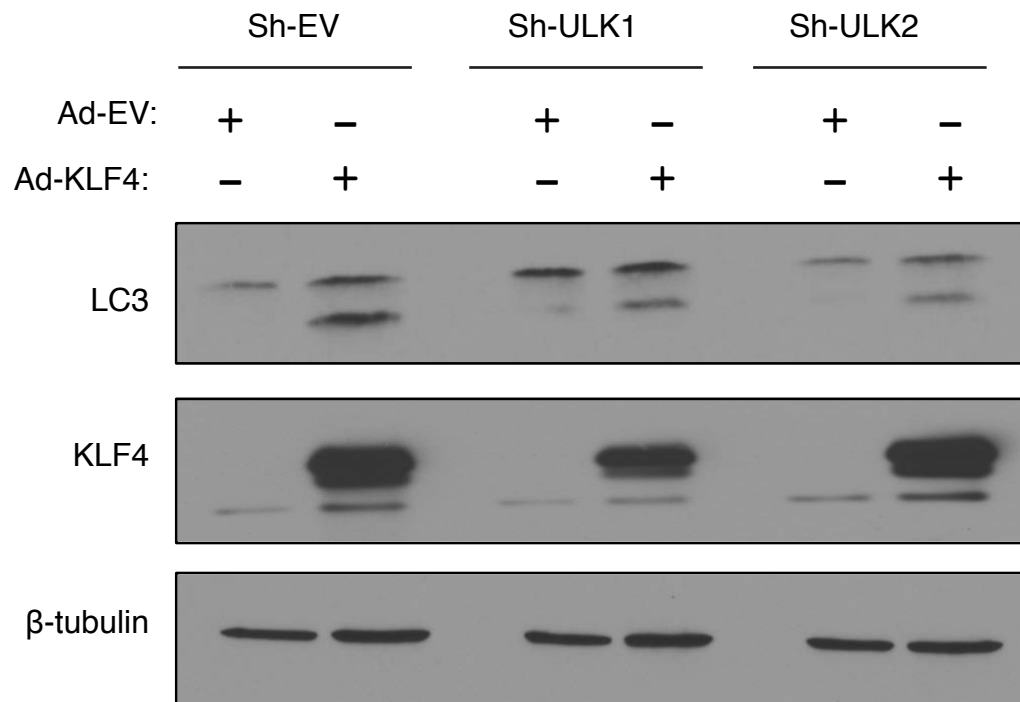
Supplementary Figure 13. KLF4 directly activates ULK1 promoter.

(A) KLF4 activated ULK1 promoter in NRVM. (B) The KLF4-mediated activation of ULK1 promoter requires the DNA binding domain (ZnF) and it is specific to KLF4. Luciferase reporter activity was assayed 24h post transfection. n=3 in each group. *p<0.05.

A**B**

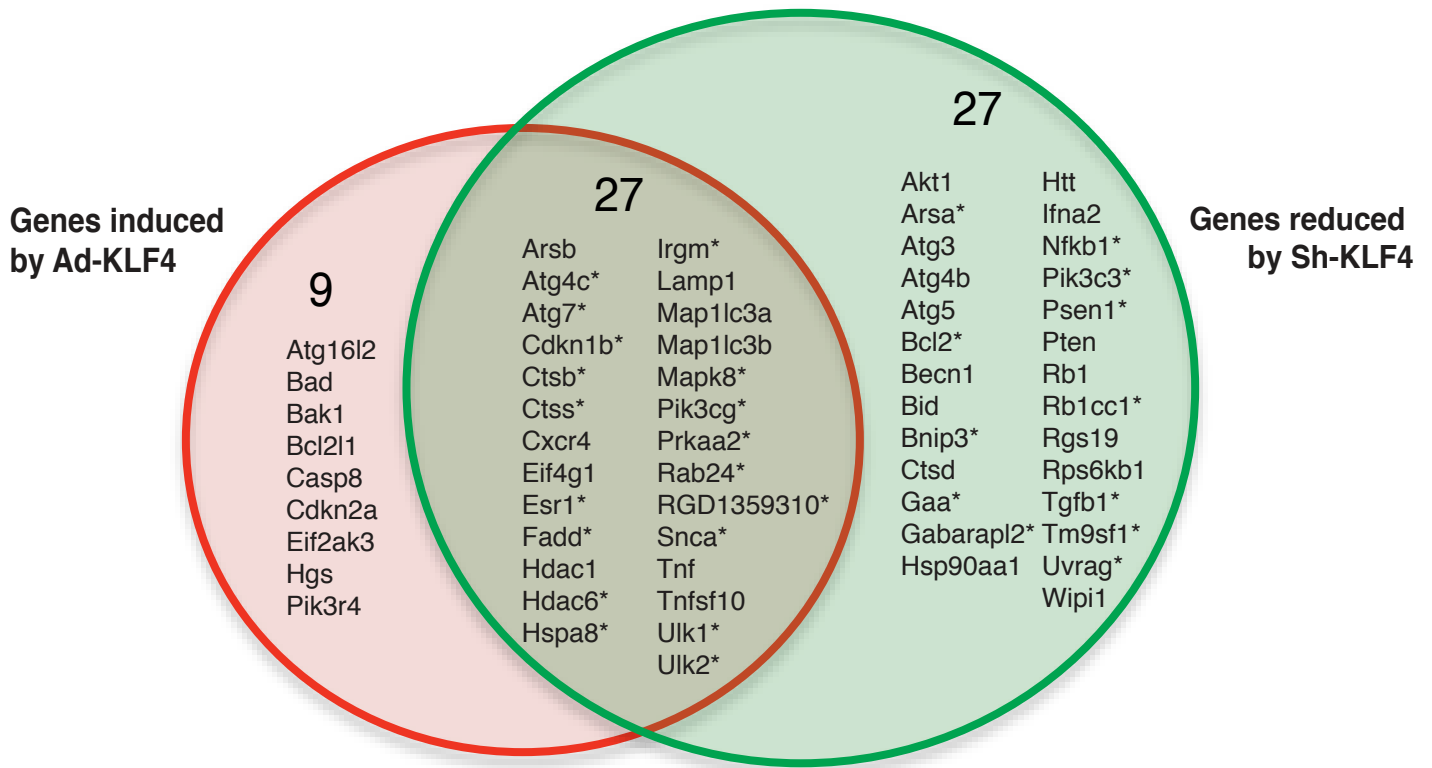
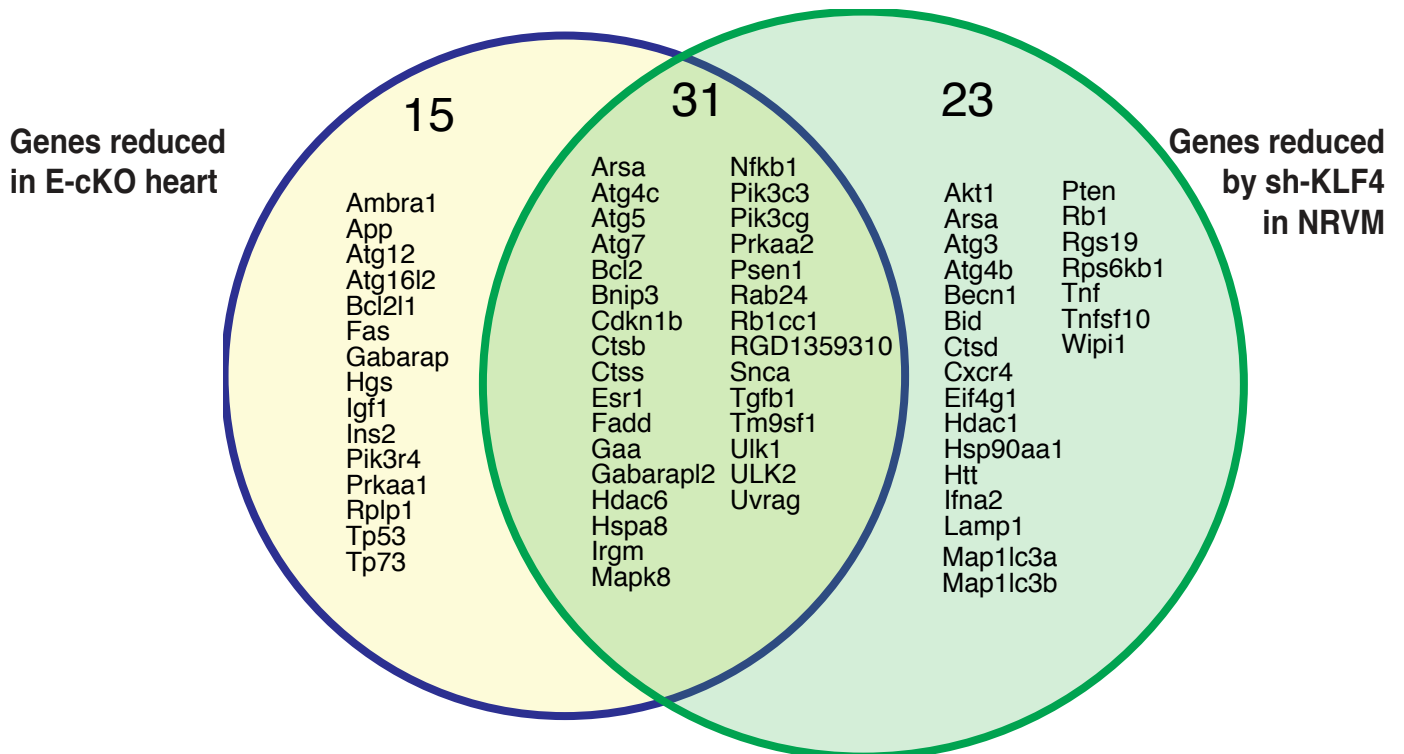
Supplementary Figure 14. KLF4-induced LC3 activation is dependent on ULK1 and ULK2.

(A) KLF4 regulates transcription of ULK1 and ULK2. (B) KLF4 binds to ULK2 proximal promoter. (C) Adenoviral knockdown of ULK1 and ULK2 in NRVM. (D) Deficiency of ULK1 or ULK2 attenuated KLF4-mediated LC3 activation. NRVMs were infected with scramble shRNA (Sh-EV) or Sh-ULK1, Sh-ULK2 adenovirus for 48 h before secondary infection with adenoviral KLF4. Cells were cultured for another 24 h before harvested for Western blot analysis. * $p < 0.05$, $n = 6$ in each group.

A**B****C****D**

Supplementary Figure 15. KLF4 regulates autophagy signaling in cardiomyocytes.

(A) Venn diagram showing genes that were significantly induced by KLF4 overexpression or reduced by KLF4 knockdown (difference over ± 1.5 fold and $p < 0.05$). NRVMs were infected with adenovirus expressing KLF4 mRNA or KLF4-targeting shRNA for 72 h. Corresponding empty viruses (Ad-EV, Sh-EV) were used as control. (B) Venn diagram showing genes that were significantly reduced in KLF4-silencing NRVM *in vitro* and in KLF4 deficient heart *in vivo* (difference over -1.5 fold and $p < 0.05$). *Asterisks indicate genes that are recapitulated *in vivo*. Total RNA was extracted, purified, reverse transcribed into cDNA and subjected to qPCR analysis with the RT² Profiler PCR Array targeting autophagy pathway (Qiagen, PAMM-084Z, PARN-084Z).

A**B**

Supplementary Table I. Substrates used for mitochondrial respiration assays.

Substrate	Check point(s)
Palmitoyl-CoA	Palmitoyl-CoA (+carnitine+Malate): requires all steps of mitochondrial fatty acid transport and oxidation (FAO), TCA and ETC.
Palmitoyl-carnitine	Palmitoyl-carnitine (+Malate): bypasses mitochondrial carnitine palmitoyl transferase 1 (CPT1) as the limiting step of fatty acid mitochondrial transport, uses long-chain acyl dehydrogenase (LCAD), and requires TCA, ETC.
Octanoyl-carnitine	Octanoylcarnitine (+Malate): bypasses CPT1, uses medium-chain acyl dehydrogenase (MCAD), and requires TCA, ETC.
Acetyl-carnitine	Acetylcarnitine (+Malate): bypasses FAO, CPT1, uses carnitine acylcarnitine translocase (CACT) and requires TCA, ETC.
Pyruvate	Pyruvate (+Malate): uses pyruvate transporter and dehydrogenase, produces NADH from TCA, and NADH is oxidized through ETC.

Supplementary Table II. Primer sequences for quantitative real-time PCR.

qPCR primers for mouse genes

Gene	Official symbol	Forward primer 5'-3'	Reverse primer 5'-3'	Official Full Name
MCAD	Acadm	cgaacacaactcgaagc	ctgctgtccgtcaactcaa	acyl-Coenzyme A dehydrogenase, medium chain
VLCAD	Acadvl	ggtggttggcctctcta	gggtaacgctaacaccaagg	acyl-CoA dehydrogenase, very long chain
ACSL1	Acs1l	aaagatggctggttacacacg	cgataatcttcaagtgccatt	acyl-CoA synthetase long-chain family member 1
sk-actin	Acta1	aatgagcgtttccgttgc	atccccgcagactccatac	actin, alpha 1, skeletal muscle
CKMT2	Ckmt2	gcaggatctggatagcagaca	ggccatctcttcttgggt	creatine kinase, mitochondrial 2
CPT1b	Cpt1b	tgctttacatcgtctccaa	ggctccagggtcagaaga	carnitine palmitoyltransferase 1b, muscle
CPT2	Cpt2	ccaagaagcagcagatgg	tagagctcaggcagggtga	carnitine palmitoyltransferase 2
CS	Cs	ggaaggctaagaacccttgg	tcactccgtcatgccatagt	citrate synthase
CYCS	Cycs	gctgctgtattatggcattga	gattctcagaaggcaagtgctta	cytochrome c, somatic
DRP1	Dnm1	tgacagtagtccacgtttca	cccatttctctgcttcaactct	dynamin 1-like
ERRalpha	Esrra	ccttccctgctggaccte	cgacaccagagcgttcaact	estrogen related receptor, alpha
FIS1	Fis1	tgggtctgtggaggatctg	attgcgtgctcttgacac	fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae)
GATA4	Gata4	ggaagacaccccaatctcg	catggccccacaattgac	GATA binding protein 4
HADHB	Hadhb	tgaaaacaagcaatgtggcta	tgaagagatacaagccatggg	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit
KLF4	Klf4	cgggaaggagagaagacact	gagttctcaccgcaacg	Kruppel-like factor 4 (gut)
LPL	Lpl	gctggtgggaatgatgtg	tggacgtgtctaggggta	ipoprotein lipase
MFN1	Mfn1	aagcataaagctcaggggatg	tgcttgaatcctctgcaa	mitofusin 1
MFN2	Mfn2	cacagtggtcagctgaaaa	ccccagaagaacacaaca	mitofusin 2
ATP6	mt-Atp6	ccataaatctaagtatagccattccac	agctttttatggttctgccaag	ATP synthase 6, mitochondrial
CO1	mt-Co1	cagaccgcaacctaacaaca	ttctgggtgcccaagaat	cytochrome c oxidase I, mitochondrial
CO2	mt-Co2	gcgcactaaatcaagcaaca	caatggcataaagctatgg	cytochrome c oxidase II, mitochondrial
CYTB	mt-Cytb	gaggttggtcgttttgg	gtttgaaagggtgggtgac	cytochrome b, mitochondrial
ND1	mt-Nd1	acacttattacaaccaagaacacat	tcataattggctatgggtcagg	NADH dehydrogenase 1, mitochondrial
ND2	mt-Nd2	ccatcaactcaatctcactctatg	gaactctgtatggtggaagg	NADH dehydrogenase 2, mitochondrial
ND5	mt-Nd5	gccaacaacatattcaacttttc	accatcatccaattagtagaaagga	NADH dehydrogenase 5, mitochondrial
NDUFB3	Ndufb3	cgggaggtcagattgctg	tttccactgctgtaatctggaag	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 3
NDUFB5	Ndufb5	ctggctatcctccagattg	cgcatcagcctcgaact	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 5
NDUFB7	Ndufb7	cccgagaagataccagctt	ggcatccatcatcttctgtt	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 7
ANP	Nppa	cacagatctgatggattcaaga	cctcatctctaccggcatc	natriuretic peptide A
BNP	Nppb	gtcagctgtttggctgtaac	agaccagcagagtcagaa	natriuretic peptide B
OPA1	Opa1	accaggagaagtagactgtgcaa	tcttcaataaacgcagaggtg	optic atrophy 1
POLRM	Polrmt	ccatgctgaactgctgga	ctcagggtgcccctctgc	polymerase (RNA) mitochondrial (DNA directed)
PPARalpha	Ppara	ctgagaccctcggggaac	aaacgtcagttcacagggaag	peroxisome proliferator activated receptor alpha
PPARdelta	Ppard	gtatgcgatggactcac	gtctgagcgcagatggact	peroxisome proliferator activator receptor delta
PGC1alpha	Ppargc1a	gaaaggccaaacagagaga	gtaaatcacacggcgtctt	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha
PGC1beta	Ppargc1b	gacgtggacgagcttctact	ctgctgttccgtcaactcaa	peroxisome proliferator-activated receptor gamma, coactivator 1 beta
CACT	Slc25a20	atccgctgcttctacaaag	tacatcccactggcaggaac	solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20
FATP1	Slc27a1	gacaagctggatcagccaag	gaggccacagaggctgttc	solute carrier family 27 (fatty acid transporter), member 1
TFAM	Tfam	caaaagatgattcgctcag	aagctgaatatgctctgtttc	transcription factor A, mitochondrial
TFB2M	Tfb2m	aaaccatcccgtcaatta	taatgcccagtcaggattc	transcription factor B2, mitochondrial
ULK1	Ulk1	ggatccatggtgctactgc	caaggcagctgattgtacc	unc-51 like kinase 1
GLUT1	Slc2a1	atgatcccagcagcaag	ccagtgttatgcccgaactgc	solute carrier family 2 (facilitated glucose transporter), member 1
LDHA	Ldha	ggcactgacgcagacaag	tgatcacctctgtaggactg	lactate dehydrogenase A
ENO1	Eno1	gaggccttagtctgct	agaatagacatggcgaatttctg	enolase 1, alpha non-neuron
PKM2	Pkm	aagggggactaccctctgg	cctcgaatagctgcaagttgg	pyruvate kinase, muscle
Gapdh	Gapdh	tggcaaatggagattgttccc	aagatggtgatggctctccc	glyceraldehyde-3-phosphate dehydrogenase
Chr6		atggaagcctgcatcatg	tcctgtgttcagcatcac	mouse chromosome 6

qPCR primers for rat genes

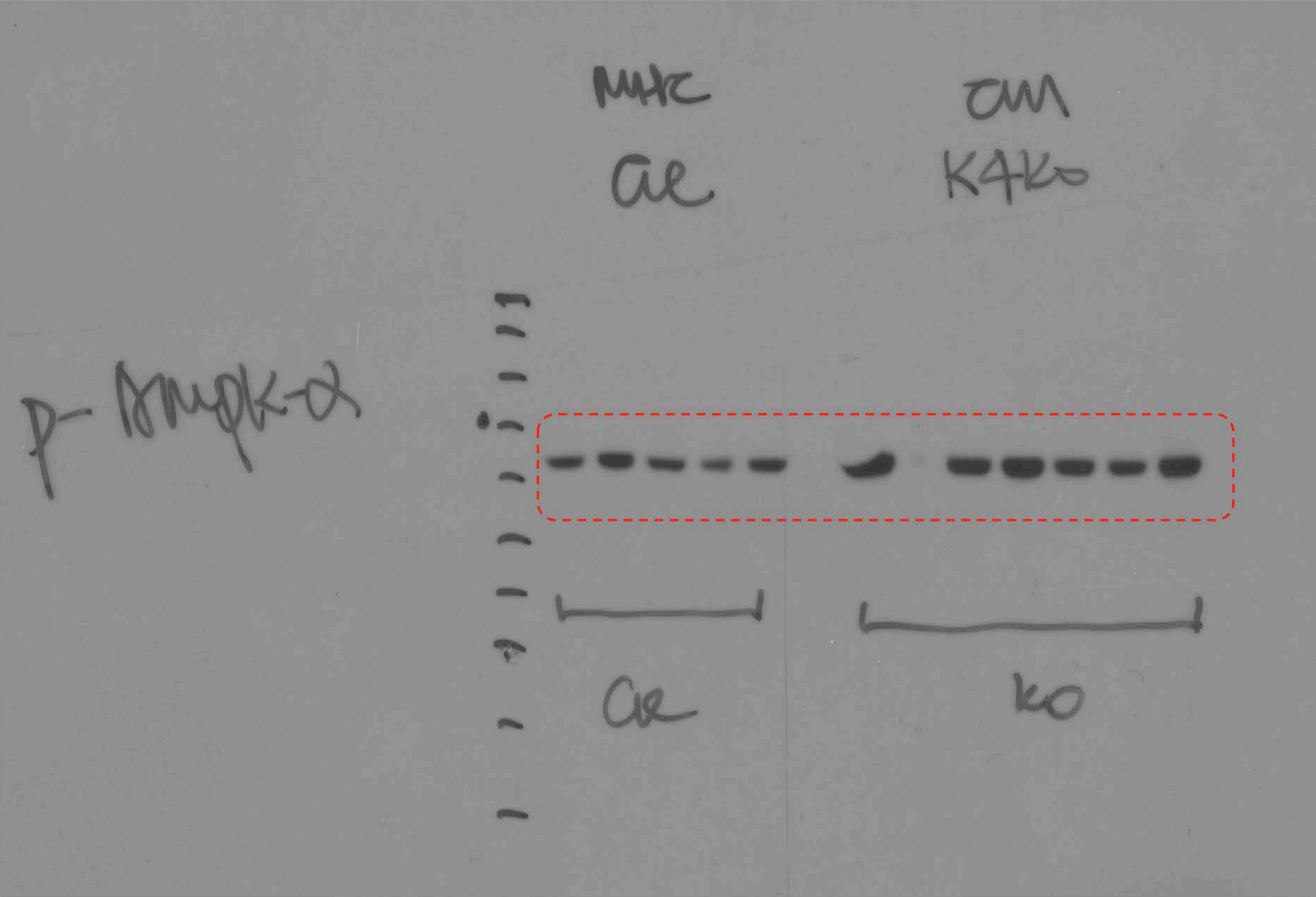
Gene	Official Symbol	Forward primer 5'-3'	Reverse primer 5'-3'	Official Full Name
Acox1	Acox1	caccttcgagggagagaaca	cgcacctggctgtagatttt	acyl-CoA oxidase 1, palmitoyl
ACSL1	Acs1l	ttacacacgggggacattg	tcctgtcgataatcttcaagggtg	acyl-CoA synthetase long-chain family member 1
ATP6	mt-Atp6	taagcatagccatcccccta	ttagttgtgtcggaagcctaga	ATP synthase 6, mitochondrial
CO3	mt-Co3	taaaccaagcccatgacc	agccggatgtaagtagaagagc	cytochrome c oxidase III, mitochondrial
COX5b	Cox5b	gaatagtgggctgcatctgtg	gggcaccaactgtaatgtgt	cytochrome c oxidase subunit Vb
CPT1b	Cpt1b	gtgactggtgggaagagtagc	ctgcttgttgctcgtgtt	carnitine palmitoyltransferase 1b, muscle
CPT2	Cpt2	agcctctcttgatgacagc	cttcccaacgccagtctc	carnitine palmitoyltransferase 2
CS	Cs	cgttgtaccttaccatccaca	tgctgcaaggacaggtaag	citrate synthase
CYCS	Cycs	gatgccacaagaacaaggt	tgggattttccaataactccat	cytochrome c, somatic
CYTB	mt-Cytb	ccctagtactattcttcccagacct	agggggtagcgggtgtat	cytochrome b, mitochondrial
ERRalpha	Esrra	cttccctgctgctcctctg	caccagggcgttaactgg	estrogen related receptor, alpha
FATP1	Slc27a1	gggtttgcaagccagaga	caagcaegcccaatgag	solute carrier family 27 (fatty acid transporter), member 1
HadhA	Hadha	ggcagctcagtcgcttctc	aaagctgtggcaaatgcag	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit
HadhB	Hadhb	ctttagtggctgctgtgc	ttggtaggctccacaatc	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), beta subunit
KLF4	Klf4	ccgtcctctccacgttc	gagttcctctcgccaacg	Kruppel-like factor 4 (gut)
LCAD	Acadl	gcagttactgggaagagcaa	ggcatgacaatatctgaatgga	acyl-CoA dehydrogenase, long chain
MCAD	Acadm	gggactagggttagcttcgag	ccgagcaattgtttaaactc	acyl-Coenzyme A dehydrogenase, medium chain
MFN1	Mfn1	caaaactgcagccaccaagt	gttggcagctcgagcaa	mitofusin 1
ND1	mt-Nd1	cctcaacctaggcataccattt	aggctcatcccgatcataga	NADH dehydrogenase 1, mitochondrial
ND2	mt-Nd2	ccattctcgcaattcatca	tttctgtttgggtctgtgt	NADH dehydrogenase 2, mitochondrial
ND4	mt-Nd4	catcagtaagccatatagccctagtc	aaagctcatggtgtctgattat	NADH dehydrogenase 4, mitochondrial
OPA1	Opa1	accaggagaagtagcgggtgc	ttctcaaatgtatcgagagggtg	optic atrophy 1
PGC1alpha	Ppargc1a	aaaggccaagcagagaga	gtaaatacacacggcgtctt	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha
PGC1beta	Ppargc1b	cttcgaggtgtttggtgagat	ctgtccttgcctctct	peroxisome proliferator-activated receptor gamma, coactivator 1 beta
POLRM	Polrmt	ctgtgctgctaaggacttct	gcagctgctgtagaaaagg	polymerase (RNA) mitochondrial (DNA directed)
PPARalpha	Ppara	tccggactaccagtacttaggg	gctggagagagggtgtctgt	peroxisome proliferator activated receptor alpha
TFB2M	Tfb2m	caaaacccatccatcaact	aatgccccagtcaggattc	transcription factor B2, mitochondrial
UCP3	Ucp3	cccctacactgtatgctgagg	agaaggaggatgatgaatcc	uncoupling protein 3 (mitochondrial, proton carrier)
ULK1	Ulk1	ccagtgctgacaggggaagg	cataaaacagccgcaaatcc	unc-51 like kinase 1
ULK2	Ulk2	cacagaacgaccaatggatg	ggcaatgccaacacaaca	unc-51 like kinase 2
GAPDH	Gapdh	gtggcattgctctcaatgaca	tccaccacctgttctgta	glyceraldehyde-3-phosphate dehydrogenase
Chr4		ctgcagcaagatgtacaccaa	tcatctgagcgtgaaaacctc	rat chromosome 4

qPCR primers for ChIP

proximal	Ppara	tcagccatctccaggtctcag	actatgctattgtggcagtaggc
proximal	Cycs	cgccggtcttagcactacc	ggtaacaccggctcttaggac
proximal	Ulk1	agccacatcgtctgttaggac	gtcggaaactcactccgactc
Distal	Ulk1	catagctagagatagttcagacctgg	gccttatagtggcggaact
proximal	Ulk2	cgacggctgcttaggaac	gaggggtgcttcaagtcag
Distal	Ulk2	cagaggactgggtttgatttc	gtcgtatcctgagactggaat
non-targeting		ctgcagcaagatgtacaccaa	tcatctgagcgtgaaaacctc

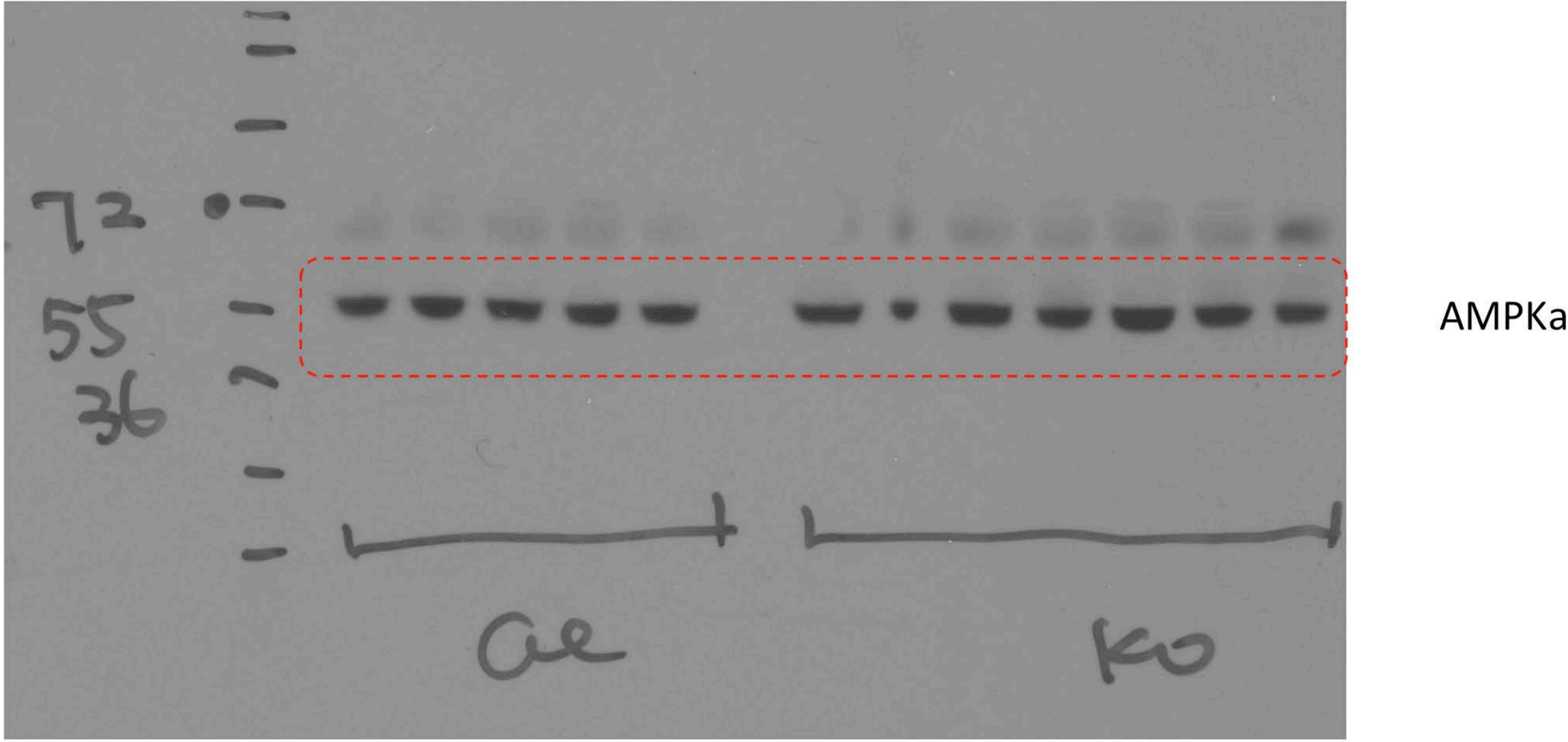
Full Unedited Gel Images

Full unedited gel for Figure 2C

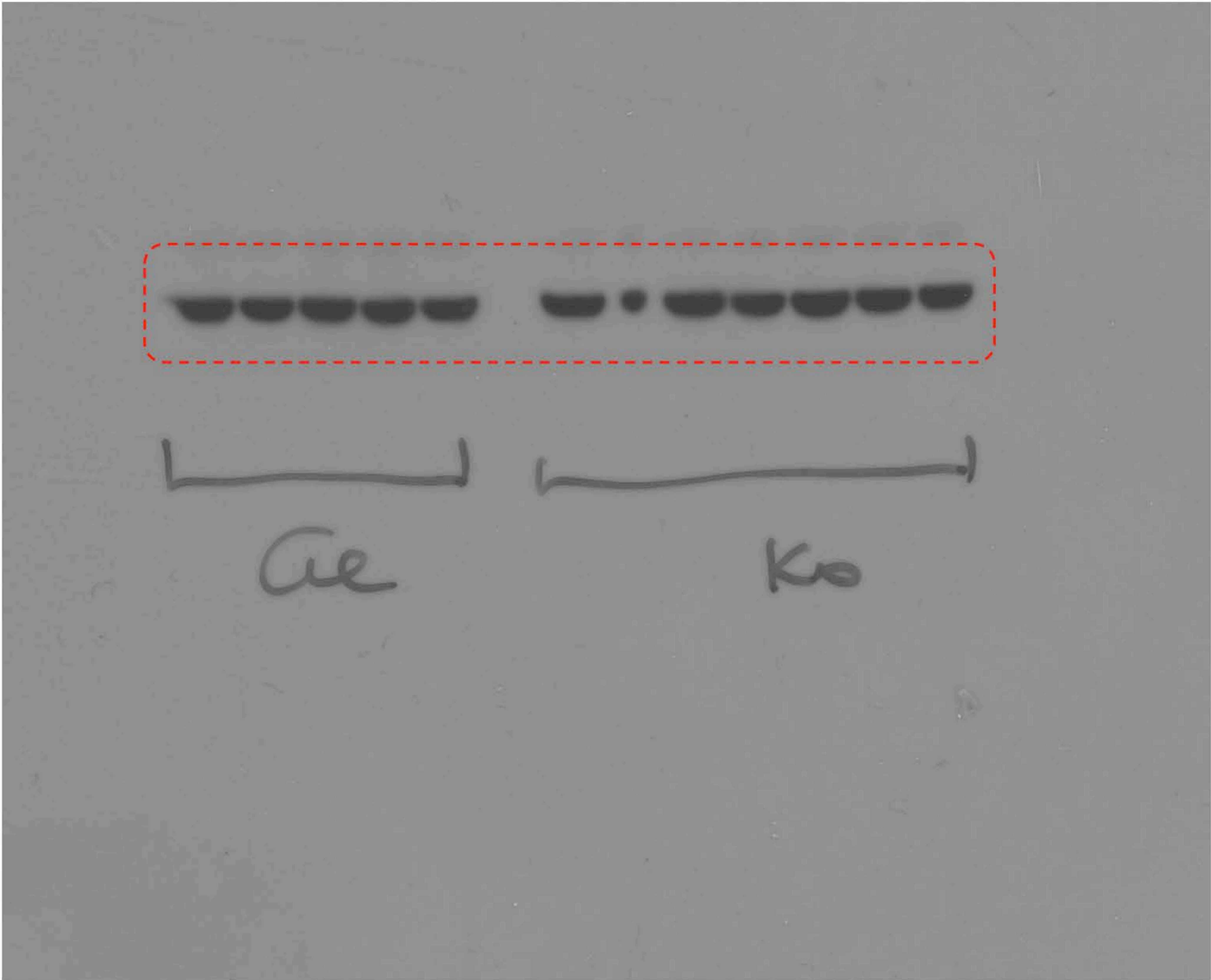


p-AMPKα

Full unedited gel for Figure 2C

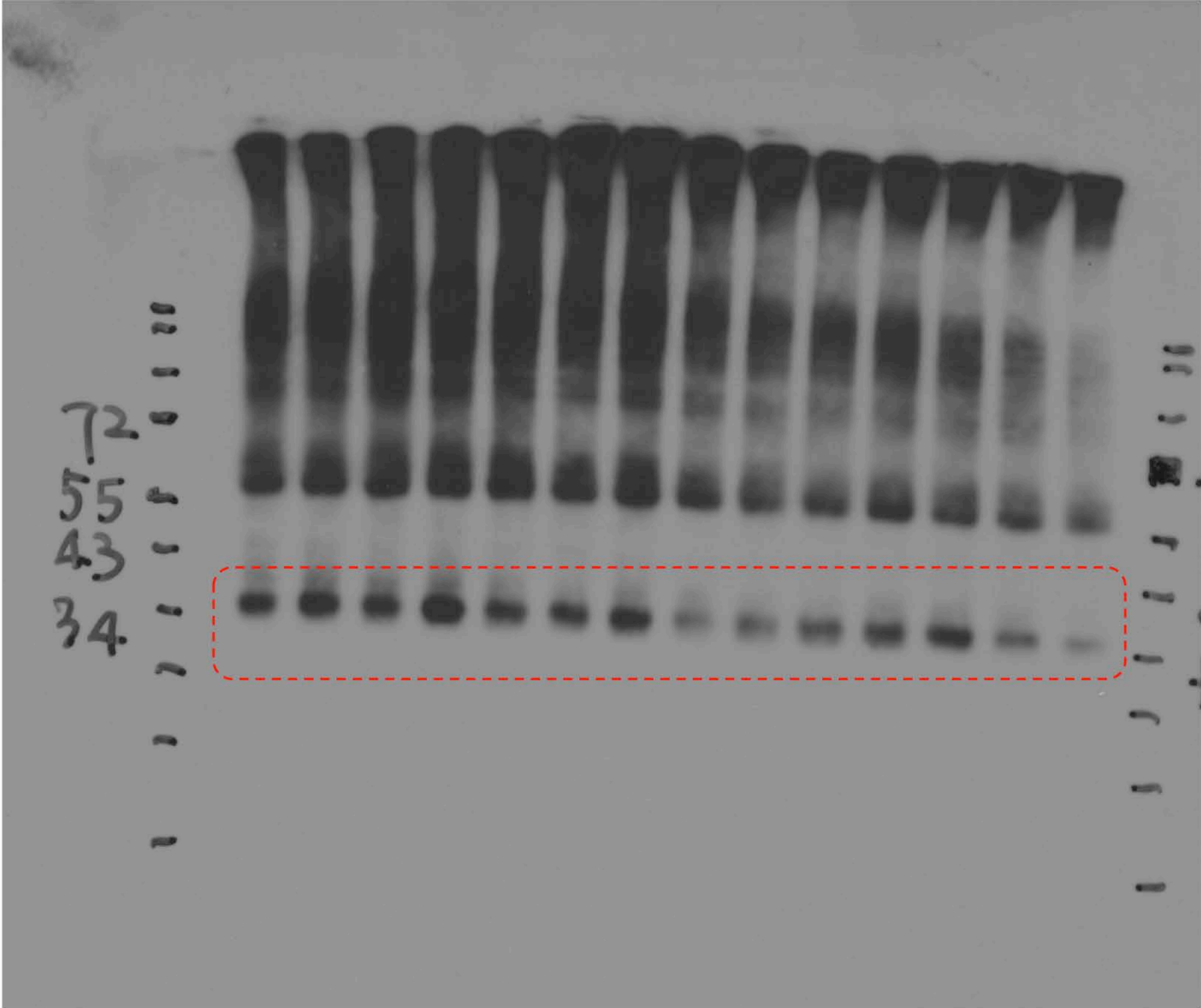


Full unedited gel for Figure 2C



beta-tubulin

Full unedited gel for Figure 4G

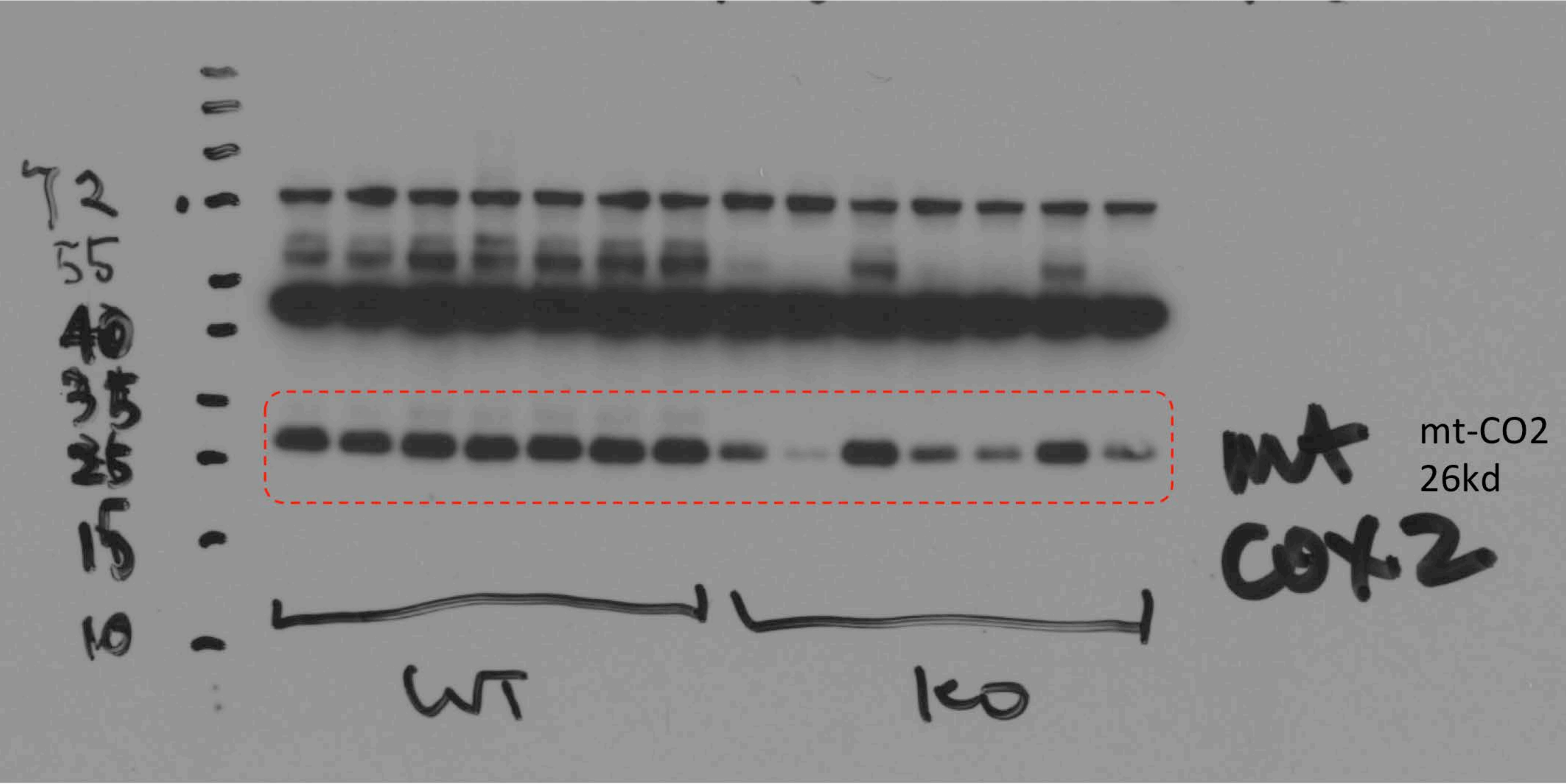


1 2 3 4 5 6 7 8 9 10 11 12 13 14

Cre 1-7

KO 8-14

Full unedited gel for Figure 4G

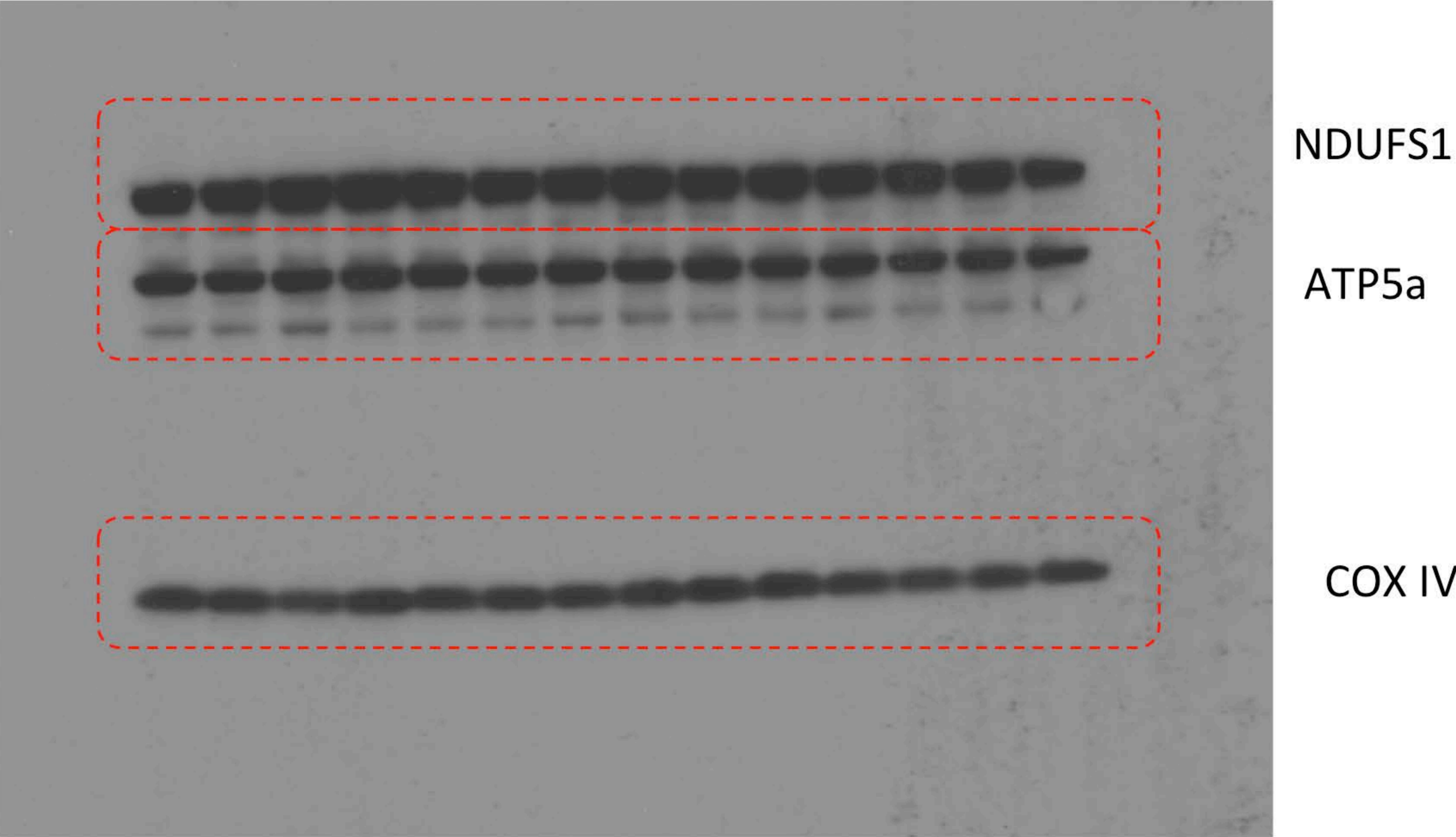


1 2 3 4 5 6 7 8 9 10 11 12 13 14

Cre 1-7

KO 8-14

Full unedited gel for Figure 4G

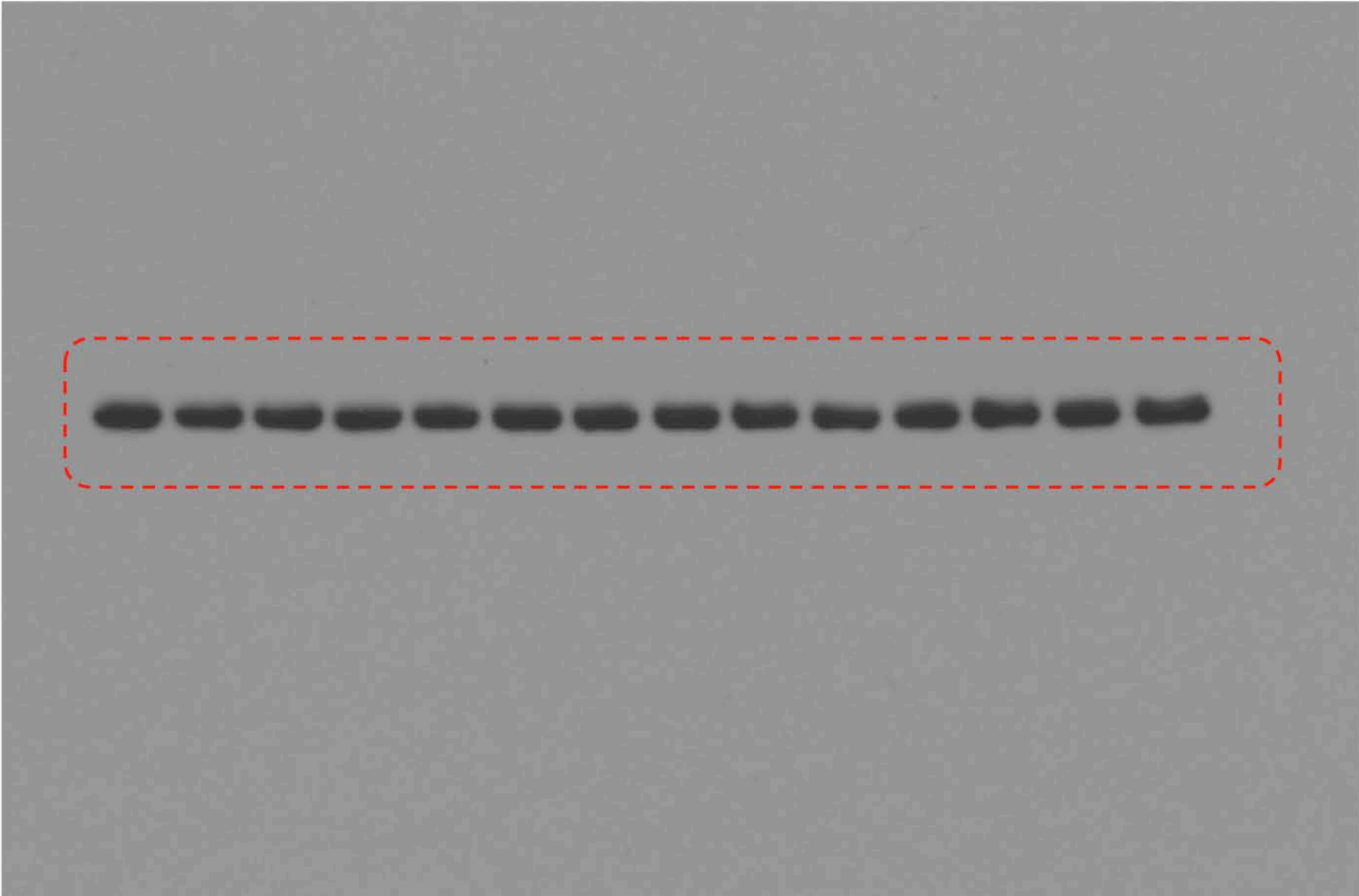


1 2 3 4 5 6 7 8 9 10 11 12 13 14

Cre 1-7

KO 8-14

Full unedited gel for Figure 4G



beta-tubulin

1 2 3 4 5 6 7 8 9 10 11 12 13 14

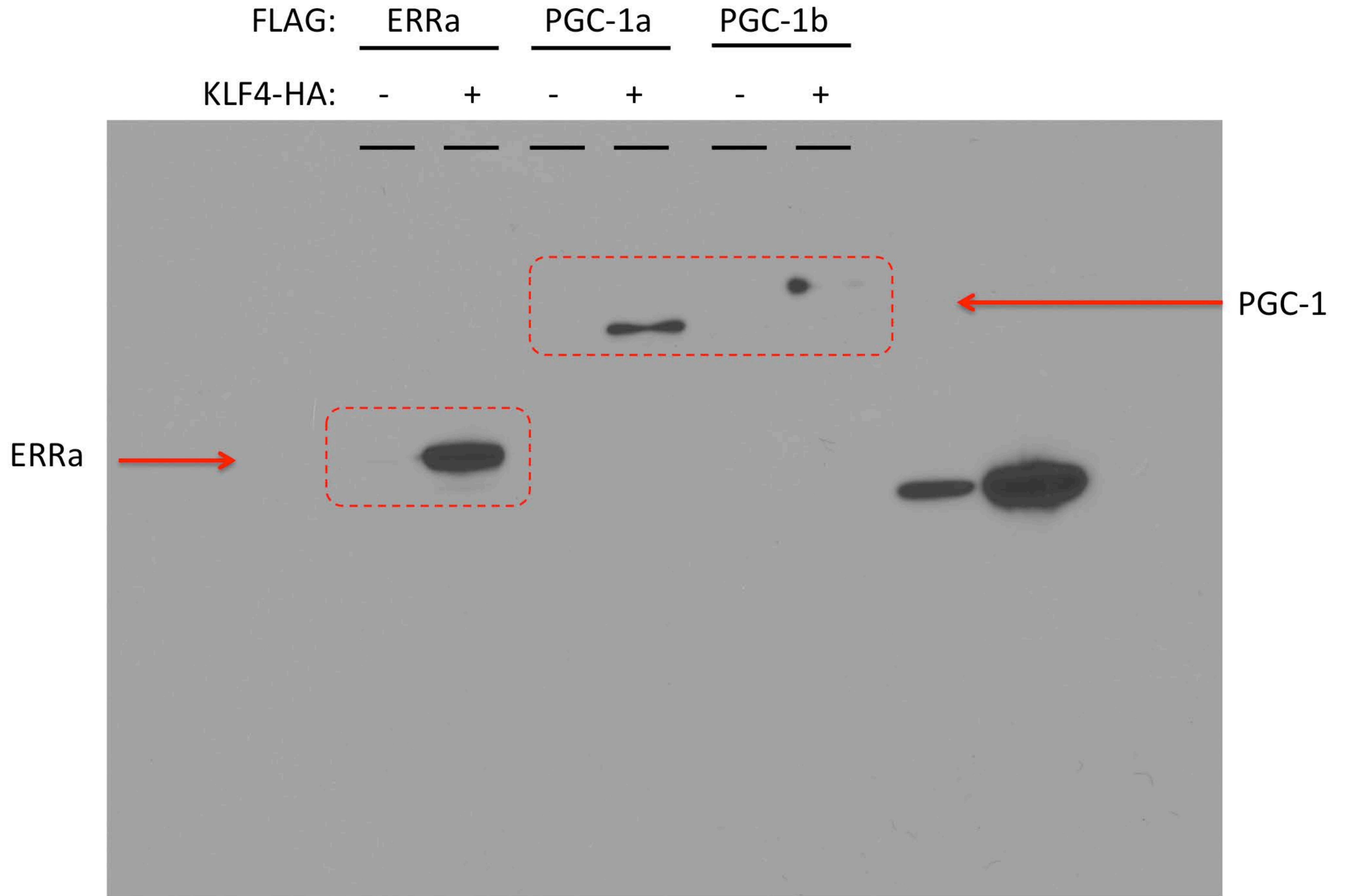
—————

—————

Cre 1-7

KO 8-14

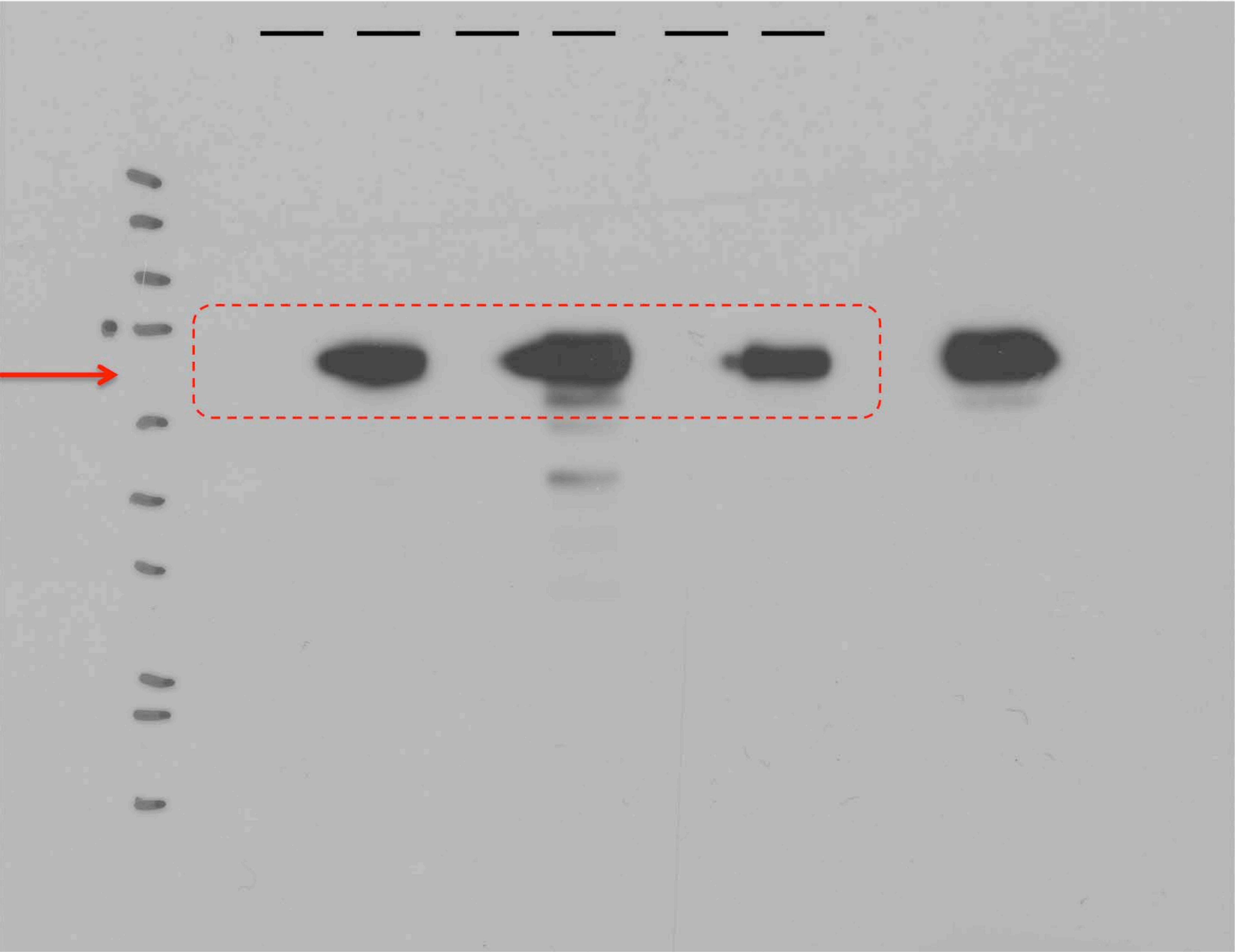
Full unedited gel for Figure 5F



IP by anti-HA, WB for FLAG

Full unedited gel for Figure 5F

FLAG:	<u>ERRa</u>		<u>PGC-1a</u>		<u>PGC-1b</u>	
KLF4-HA:	-	+	-	+	-	+

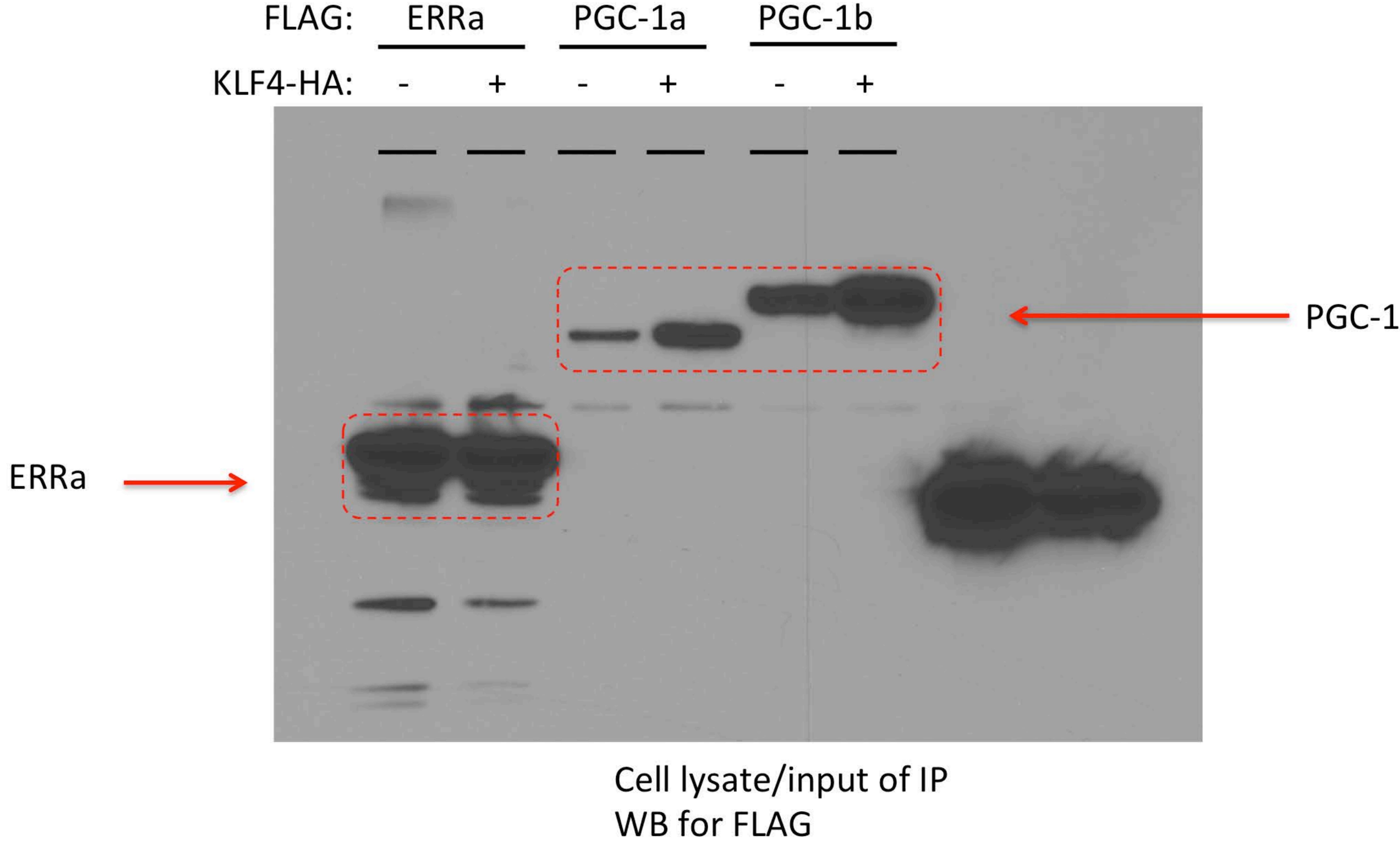


KLF4

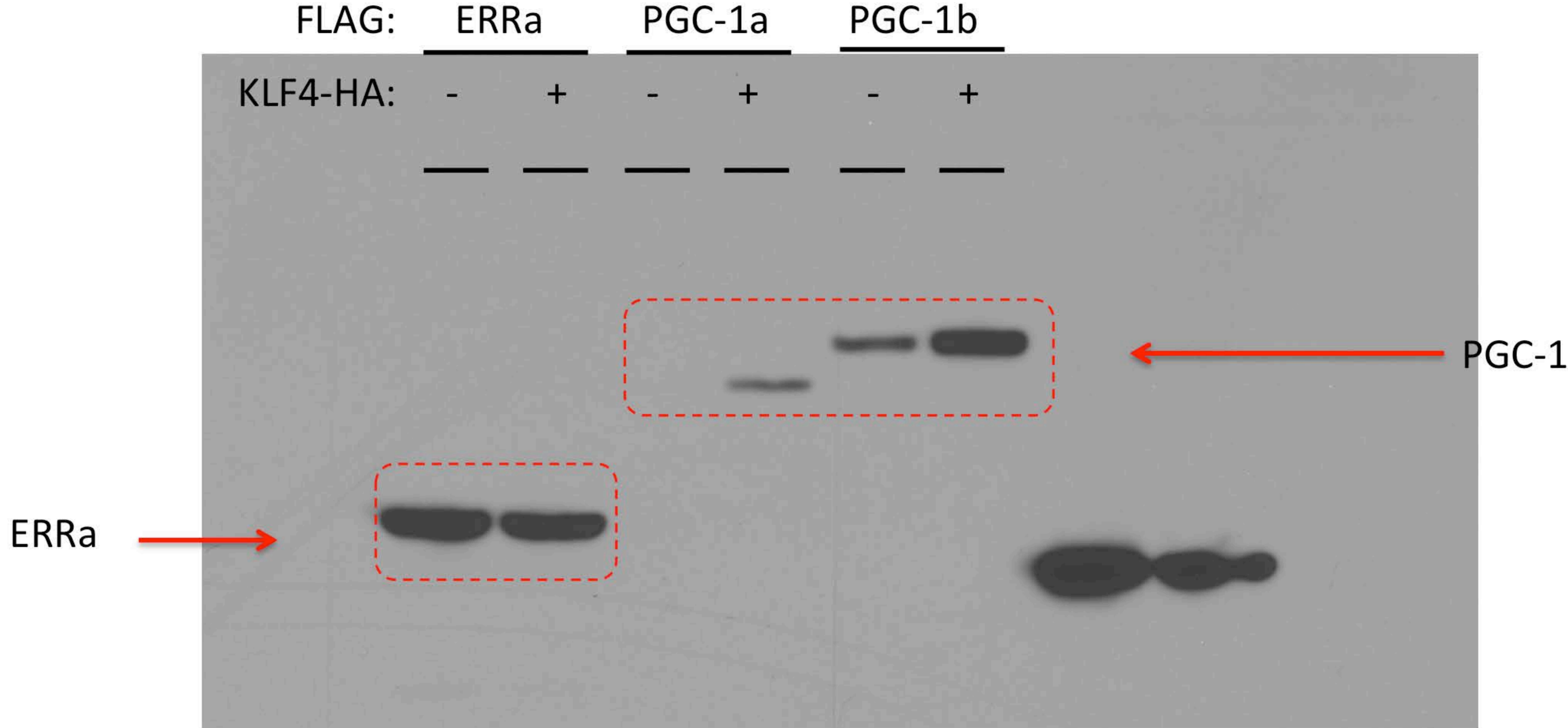


IP by anti-HA, WB for HA

Full unedited gel for Figure 5F

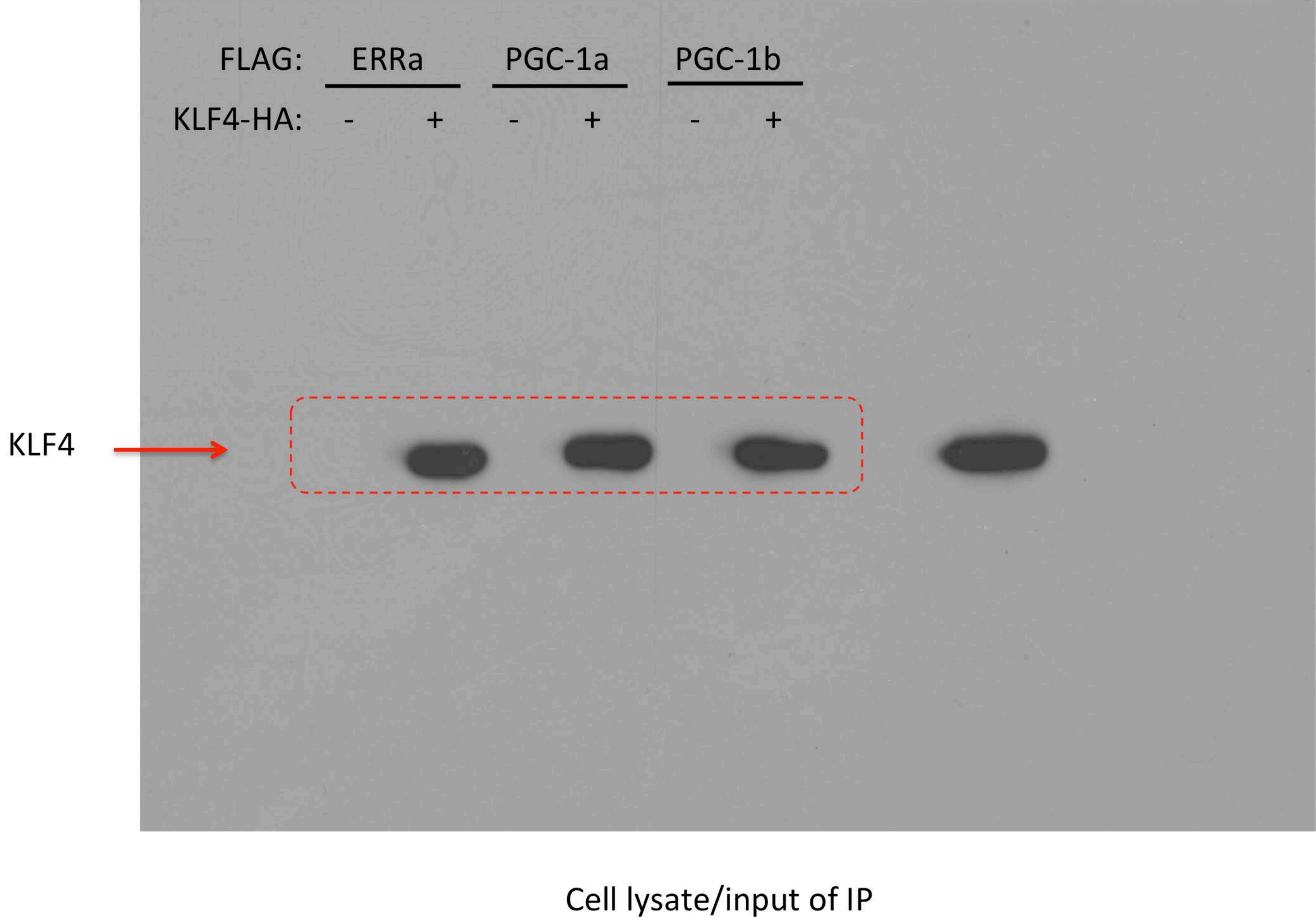


Full unedited gel for Figure 5F



Cell lysate/input of IP
Lighter exposure

Full unedited gel for Figure 5F



Full unedited gel for Figure 5G

MW:

2500

10 kD

Lane:

1	2	3	4	5	6	7	8	9	10	11	12
---	---	---	---	---	---	---	---	---	----	----	----

KLF4

70

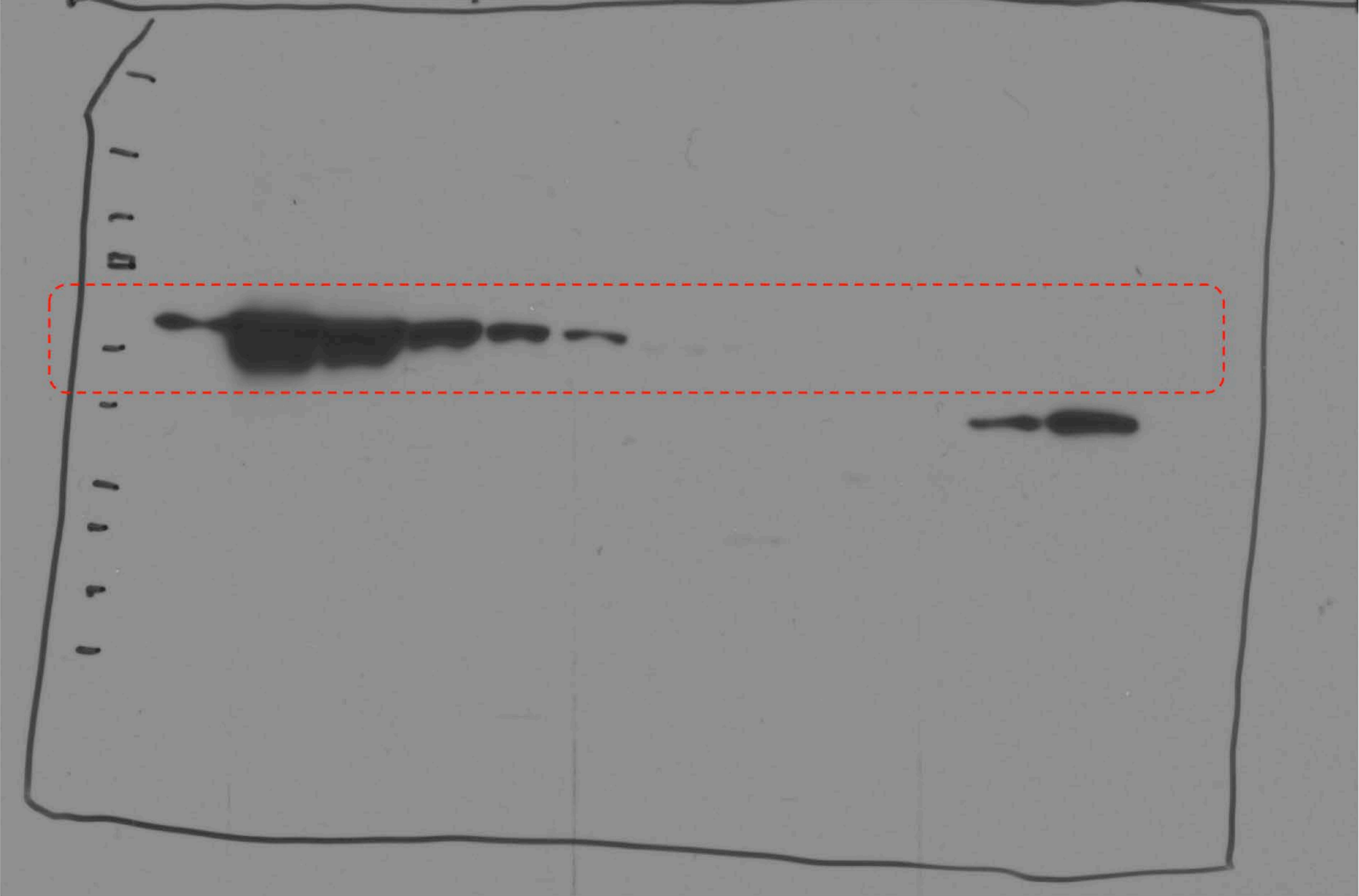


Full unedited gel for Figure 5G

MW: **2500** 10 kD

Lane:	1	2	3	4	5	6	7	8	9	10	11	12
-------	---	---	---	---	---	---	---	---	---	----	----	----

ERRa



Full unedited gel for Figure 5G

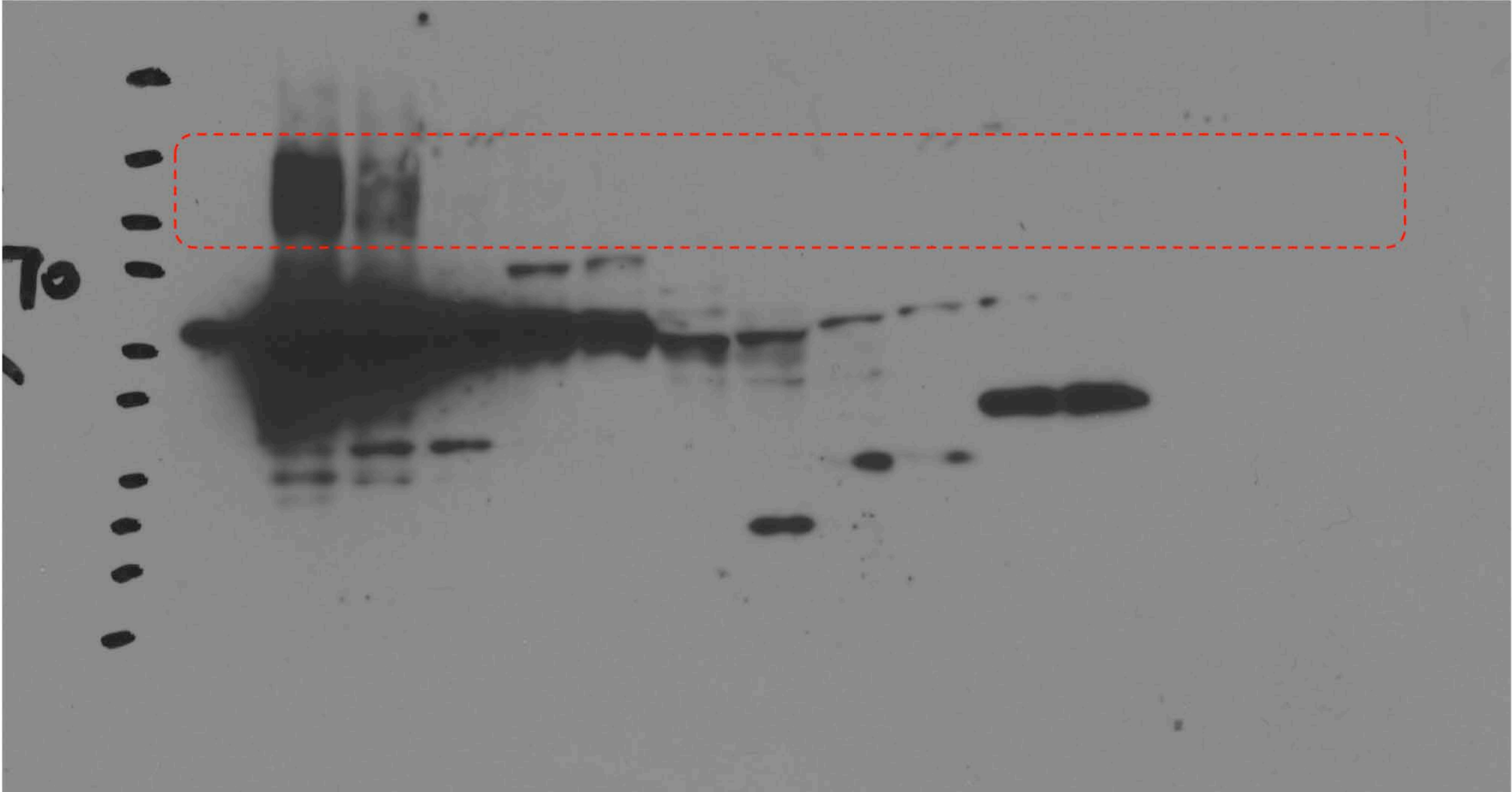
MW: **2500**

10 kD

Lane:

1	2	3	4	5	6	7	8	9	10	11	12
---	---	---	---	---	---	---	---	---	----	----	----

PGC-1a



Full unedited gel for Figure 6B

HA IP fraction

ERRa-FLAG:

+

+

+

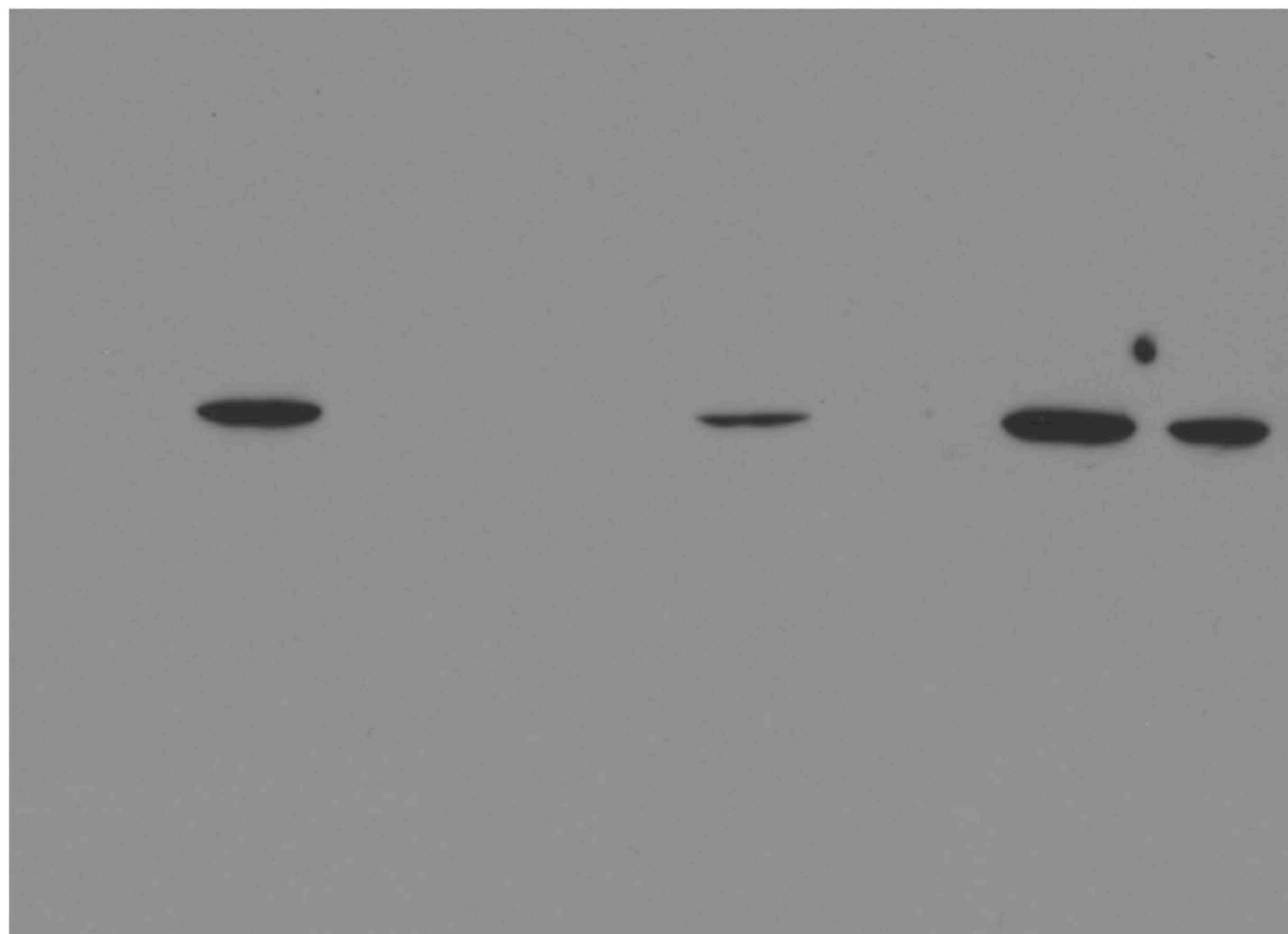
+

+

+

+

+



ERRa-FLAG

KLF4:

Vector

WT(1-479)

(1-397)

(1-118)

(118-479)

(118-397)

$\Delta(181-384)$

$\Delta(91-117)$

Full unedited gel for Figure 6B

HA IP fraction

ERRa-FLAG:

+

+

+

+

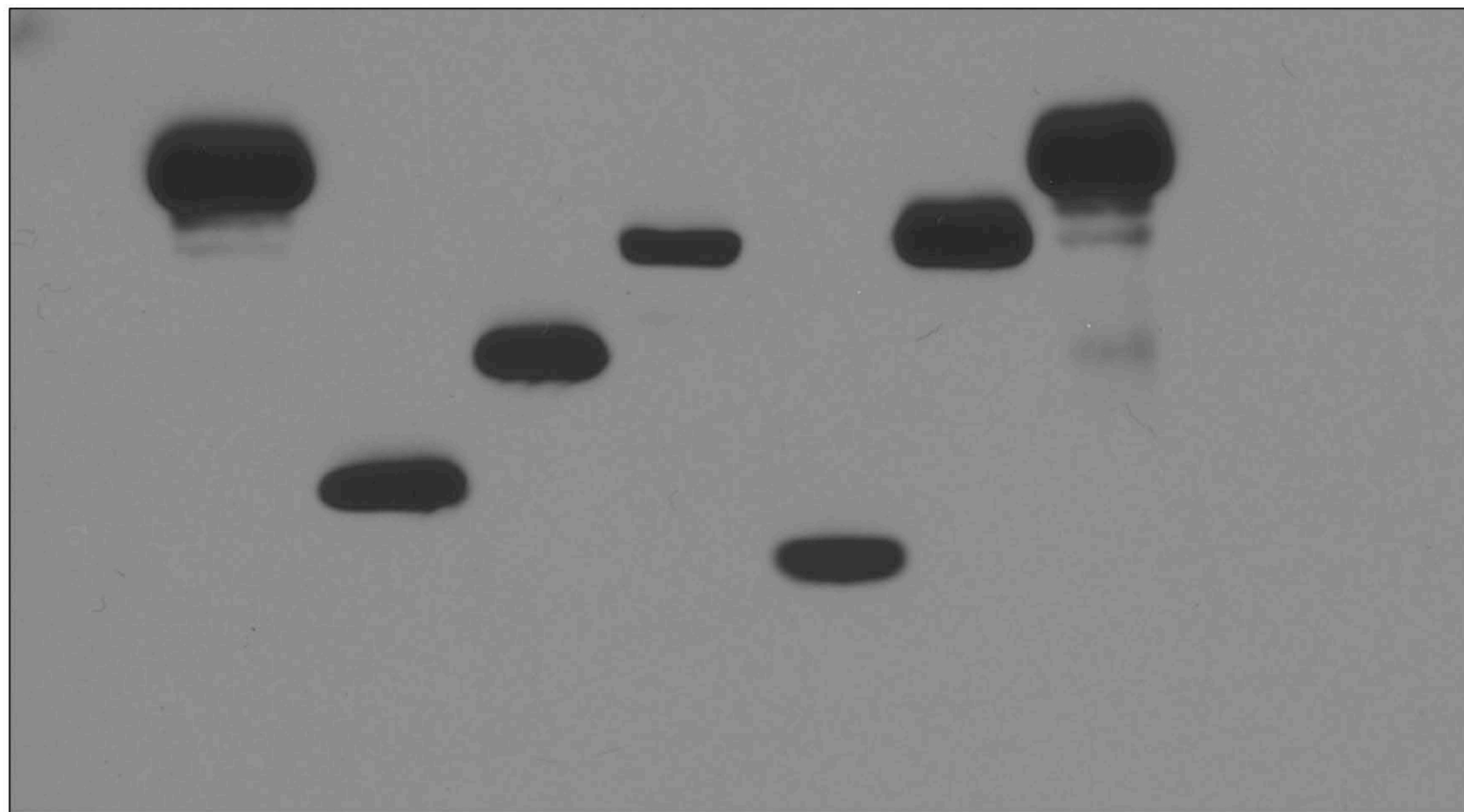
+

+

+

+

HA-KLF4
mutants



KLF4:

Vector

WT(1-479)

(1-397)

(1-118)

(118-479)

(118-397)

Δ(181-384)

Δ(91-117)

Full unedited gel for Figure 6C

Cell lysate/IP input

ERRa-FLAG:

+

+

+

+

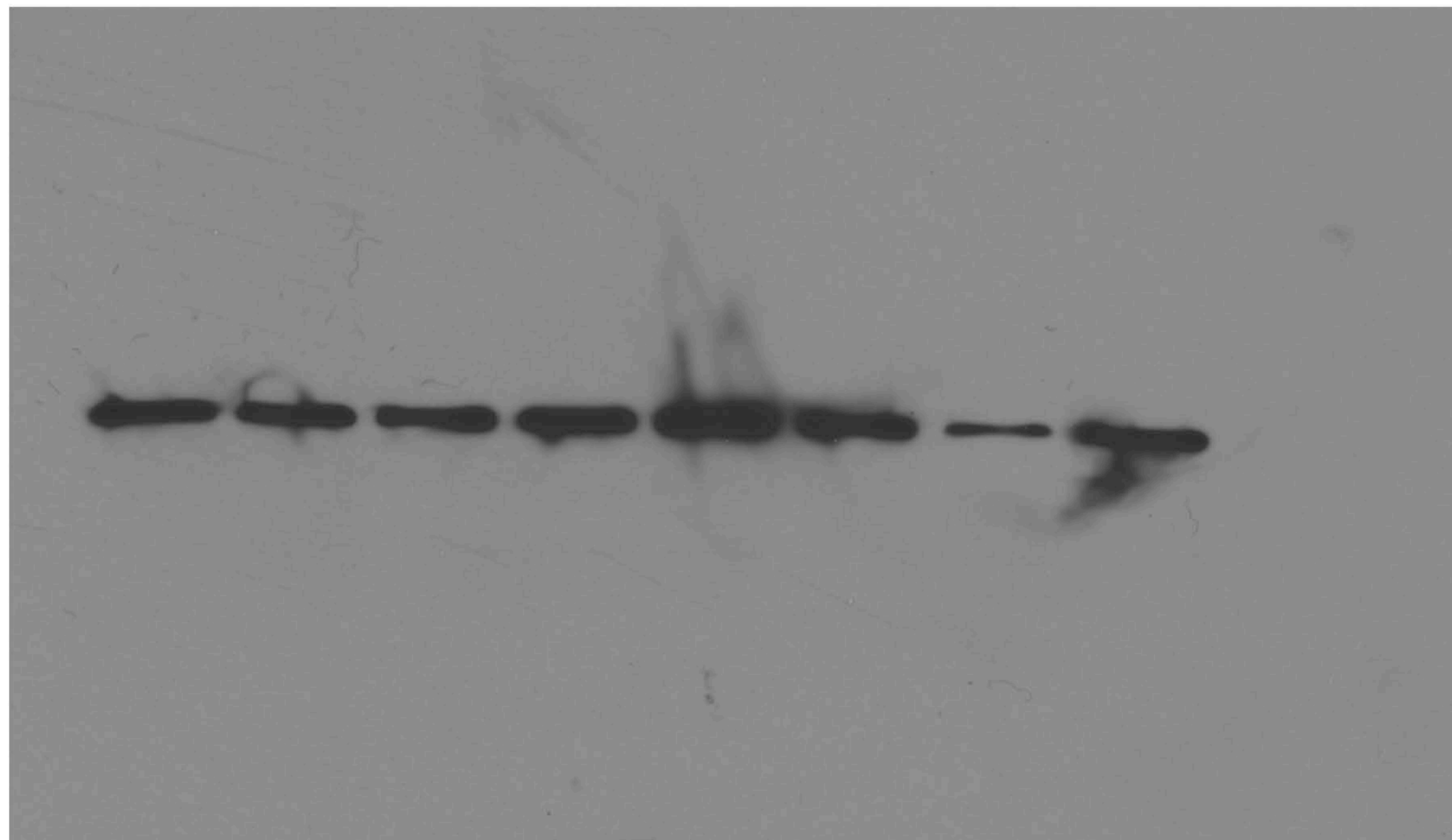
+

+

+

+

ERRa-FLAG



KLF4:

Vector

WT(1-479)

(1-397)

(1-118)

(118-479)

(118-397)

Δ (181-384)

Δ (91-117)

Full unedited gel for Figure 6C

Cell lysate/IP input

ERRa-FLAG:

+

+

+

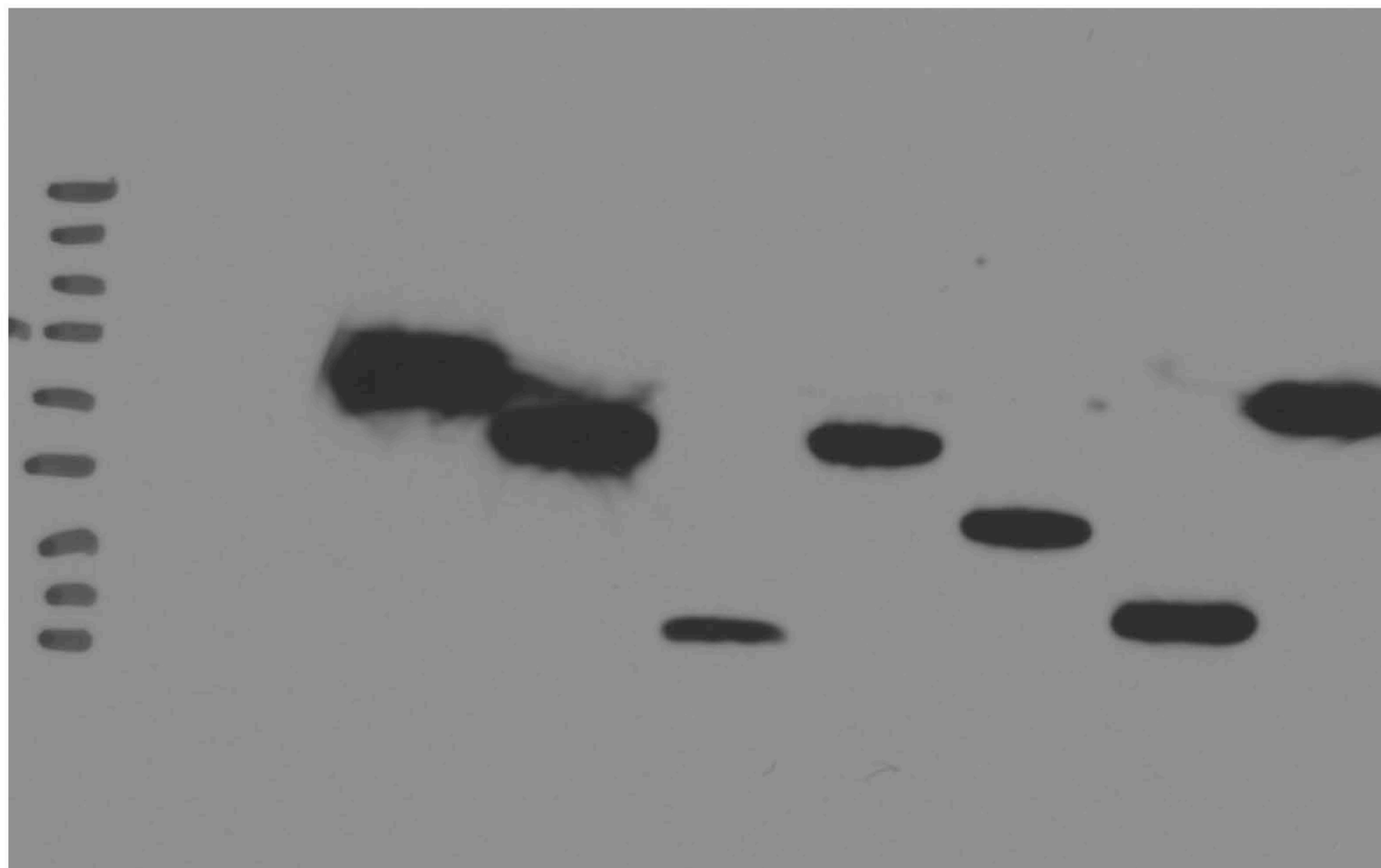
+

+

+

+

+



HA-KLF4
mutants

KLF4:

Vector

WT(1-479)

(1-397)

(1-118)

(118-479)

(118-397)

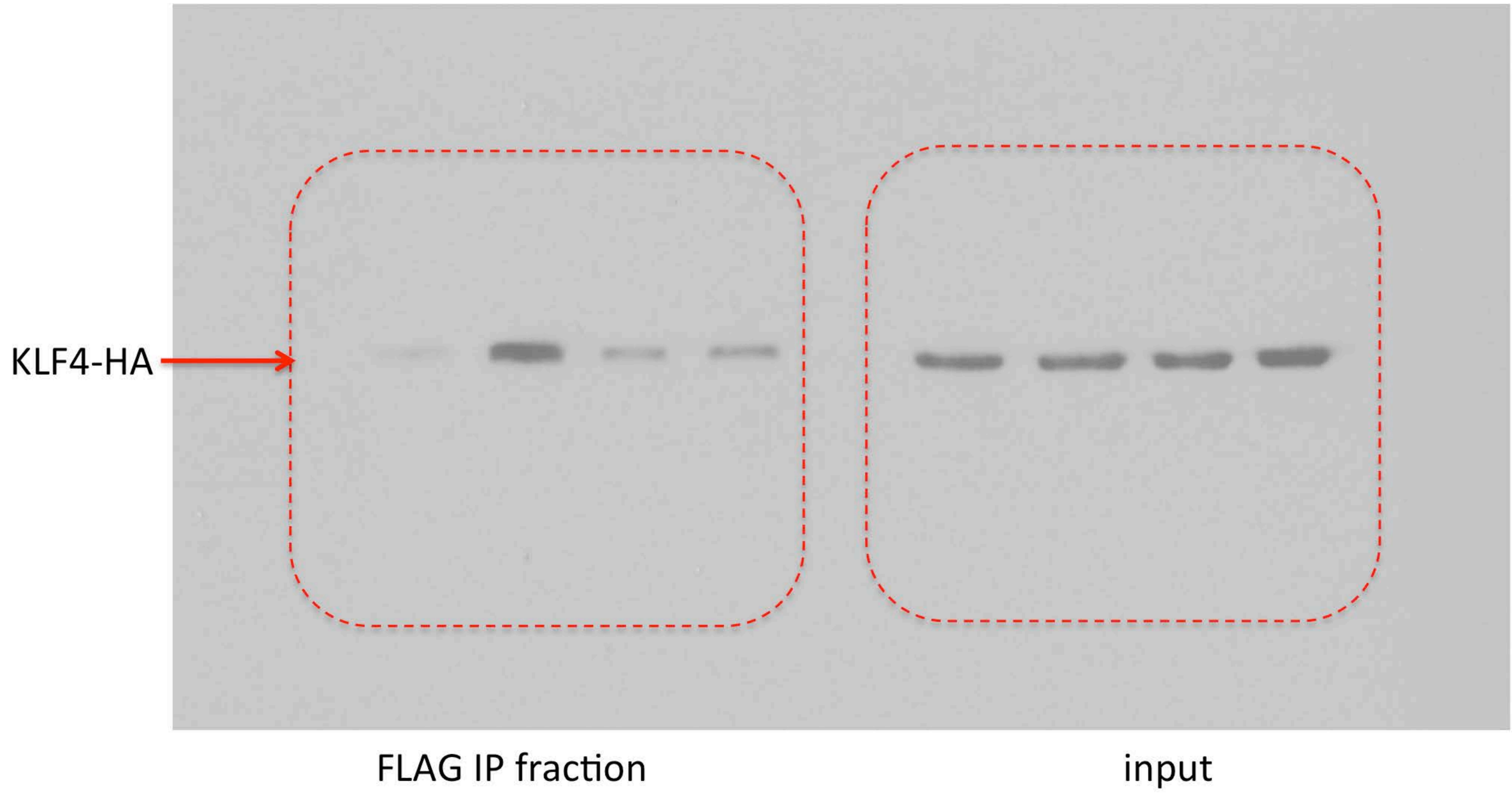
Δ (181-384)

Δ (91-117)

Full unedited gel for Figure 6E

FLAG-ERR α :
Control vector ERR α ERR α Δ 19 ERR α Δ 213 Control vector ERR α ERR α Δ 19 ERR α Δ 213

HA-KLF4: + + + + + + + + +



Full unedited gel for Figure 6E

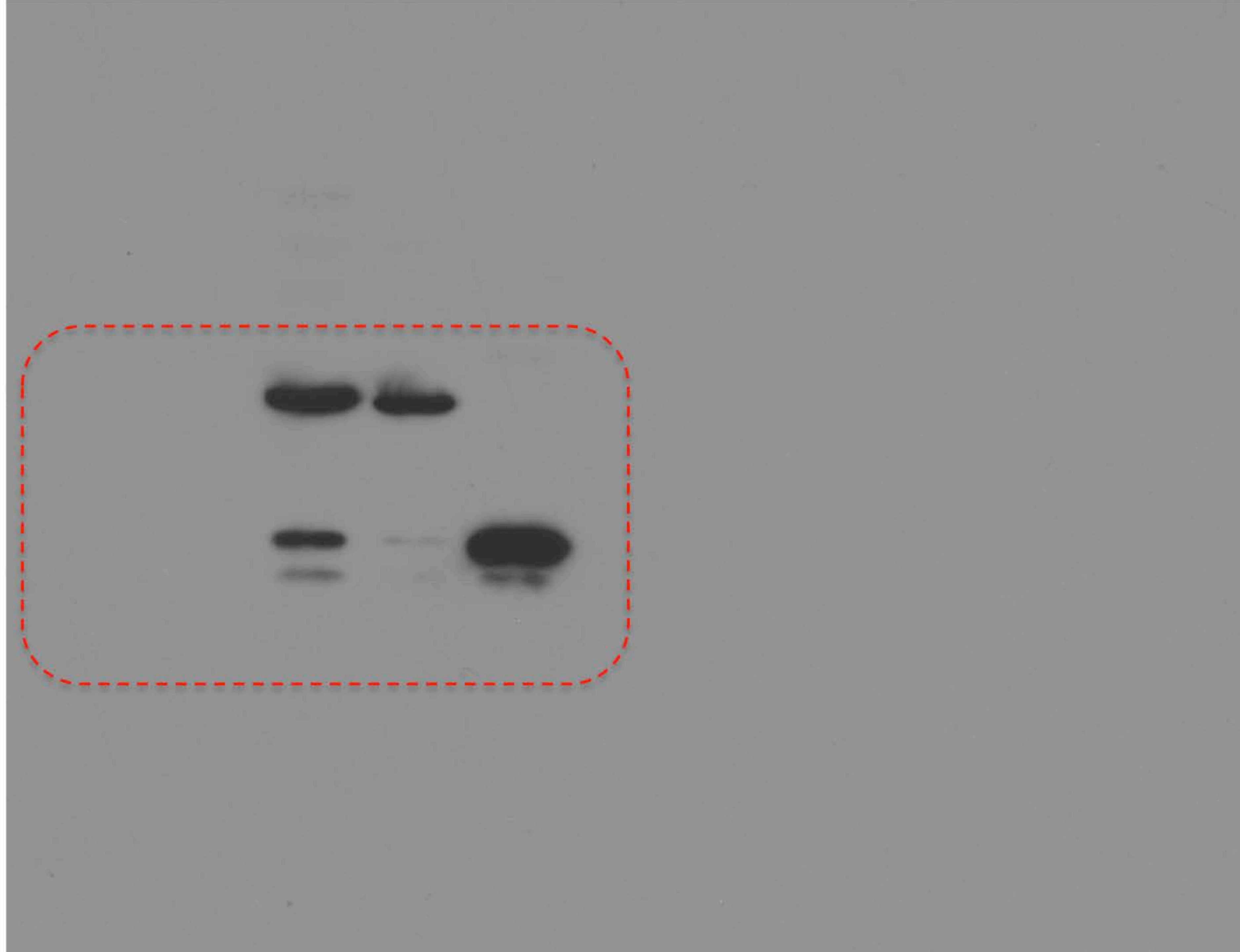
FLAG-ERR α :

Control vector
ERR α
ERR α Δ 19
ERR α Δ 213

HA-KLF4:

+ + + +

FLAG-tagged
ERRs mutants



FLAG IP fraction

Full unedited gel for Figure 6E

FLAG-ERR α :

Control vector
ERR α
ERR α Δ 19
ERR α Δ 213

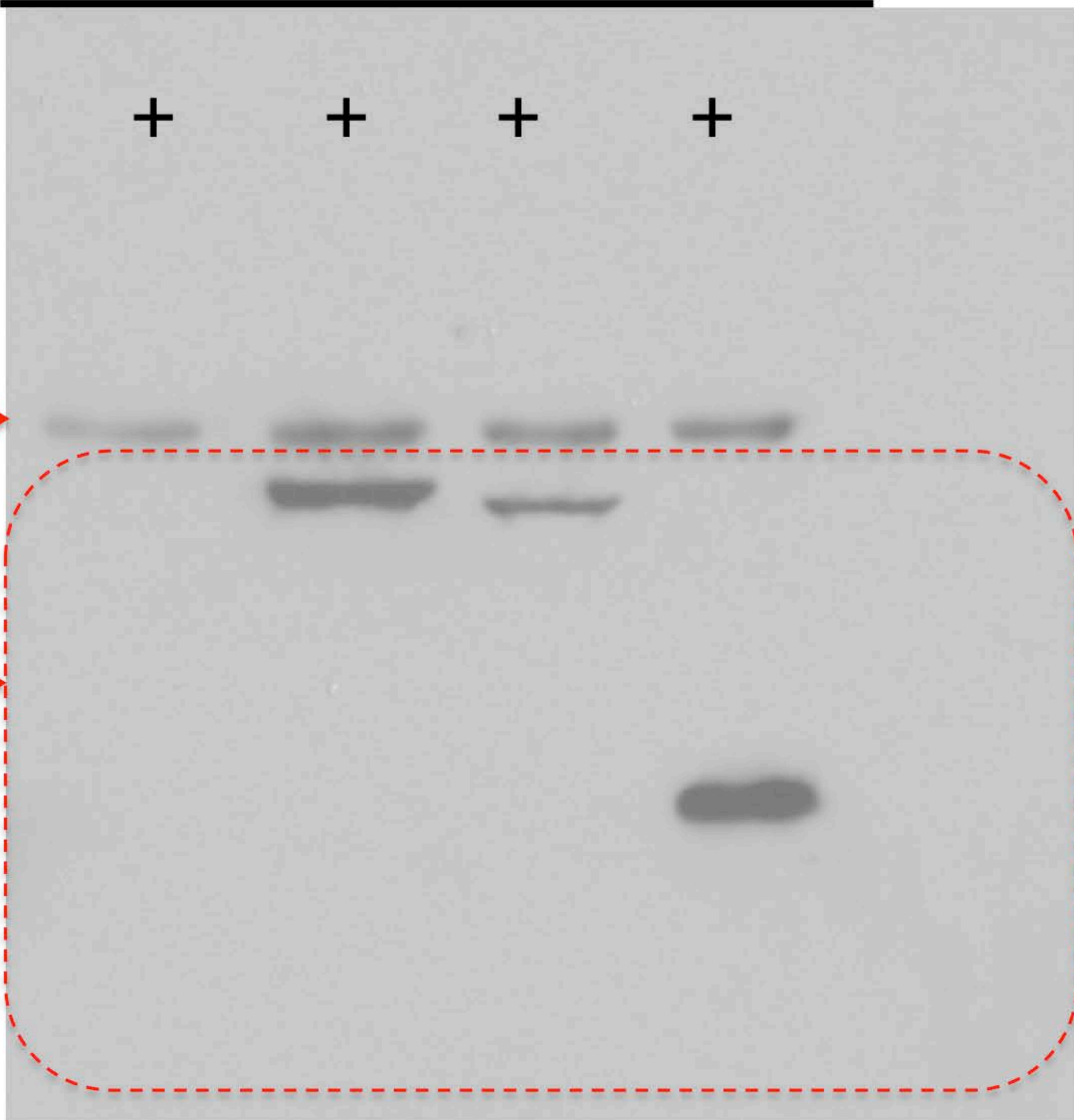
HA-KLF4:

+ + + +

KLF4-HA 

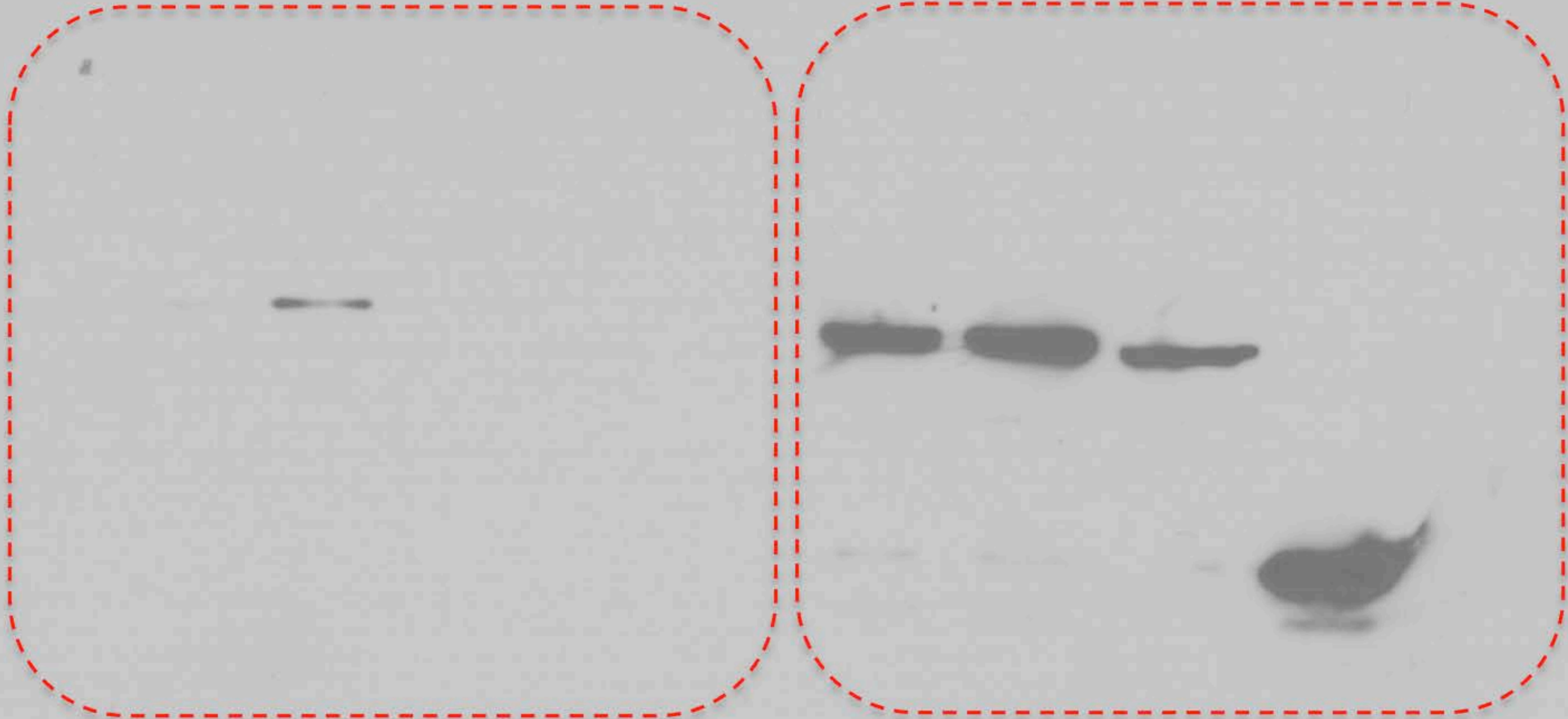
FLAG-tagged
ERRs mutants 

input



Full unedited gel for Figure 6F

IP: by endo-k9
with FLAG



IP
FLAG
ERR

input:
FLAG-ERR

Full unedited gel for Figure 6F

FLAG-ERR α :

Control vector

ERR α

ERR α Δ 19

ERR α Δ 213

Control vector

ERR α

ERR α Δ 19

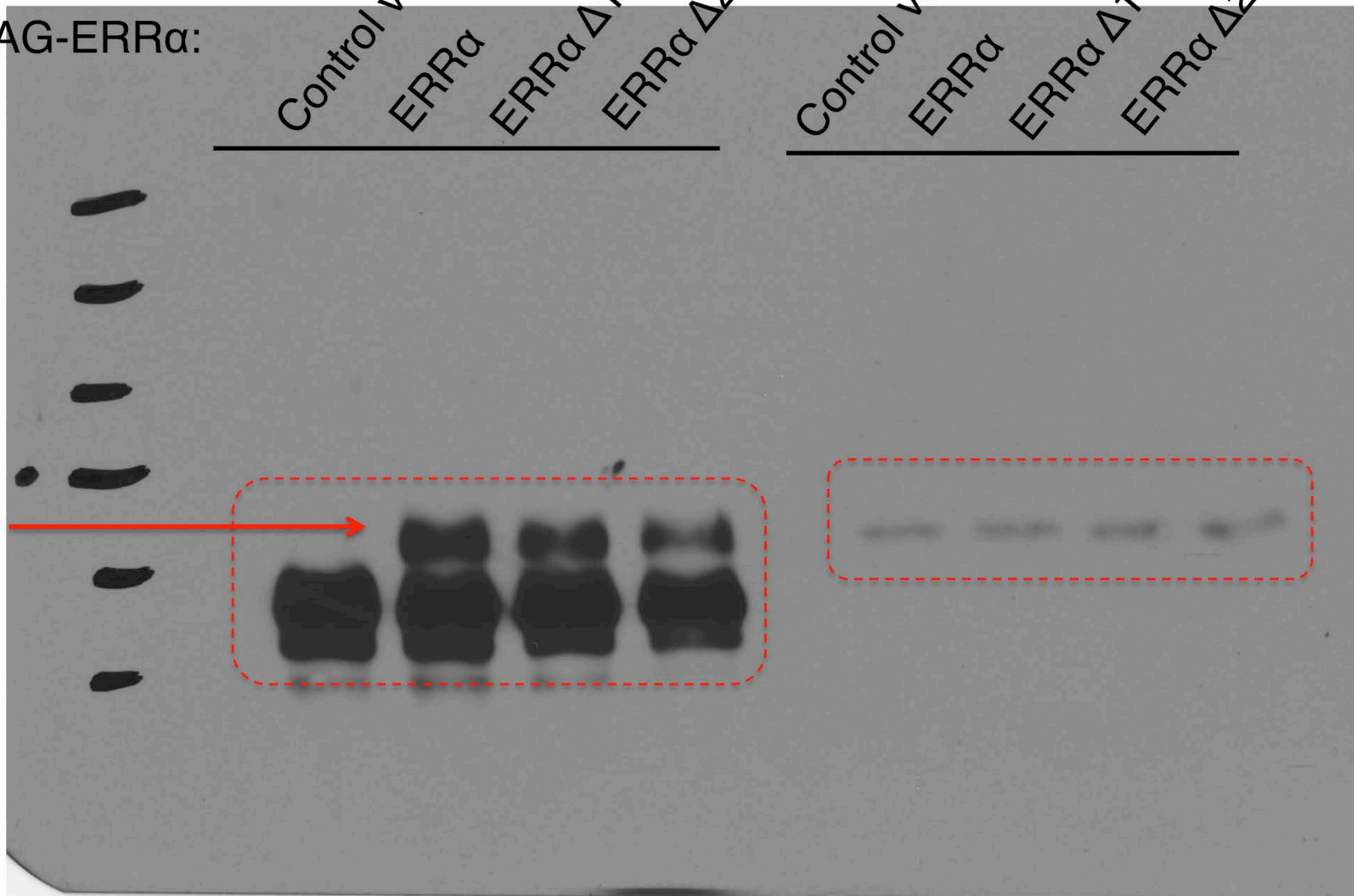
ERR α Δ 213

KLF4

KLF4

FLAG IP fraction

input



Full unedited gel for Figure 6F

FLAG-ERR α :

Control vector

ERR α

ERR α Δ 19

ERR α Δ 213

Control vector

ERR α

ERR α Δ 19

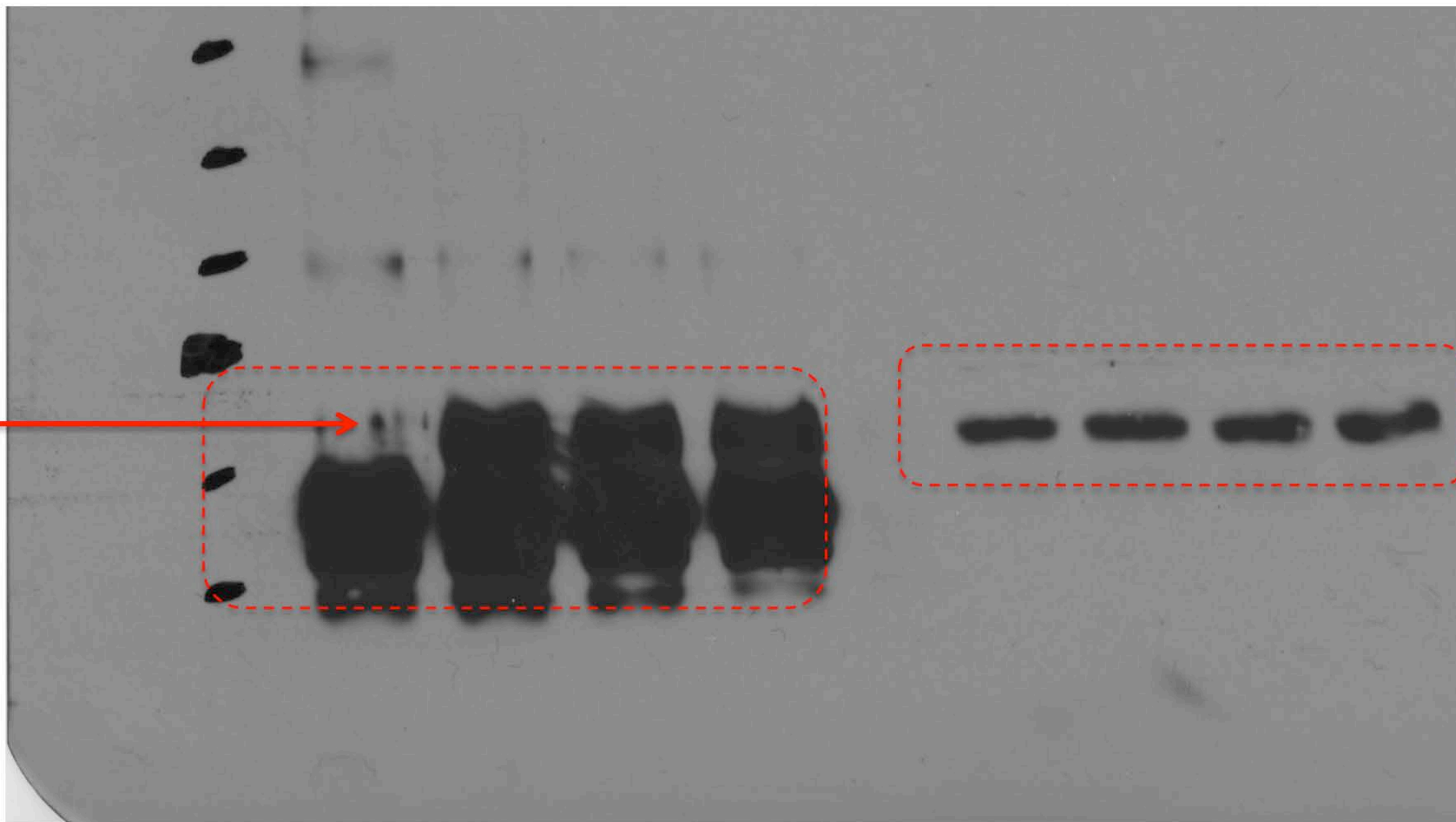
ERR α Δ 213

KLF4

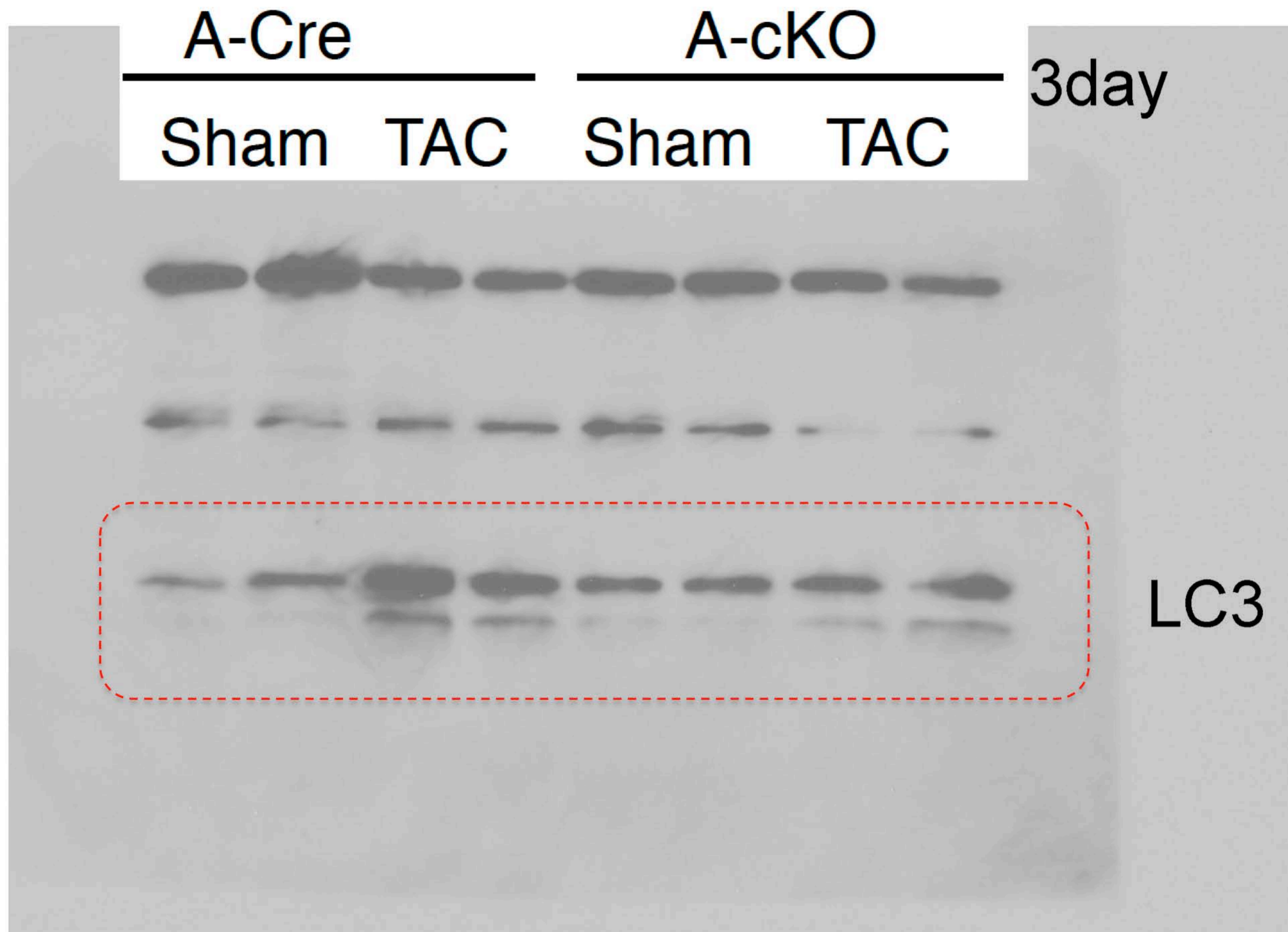
KLF4

FLAG IP fraction

input



Full unedited gel for Figure 8A-top



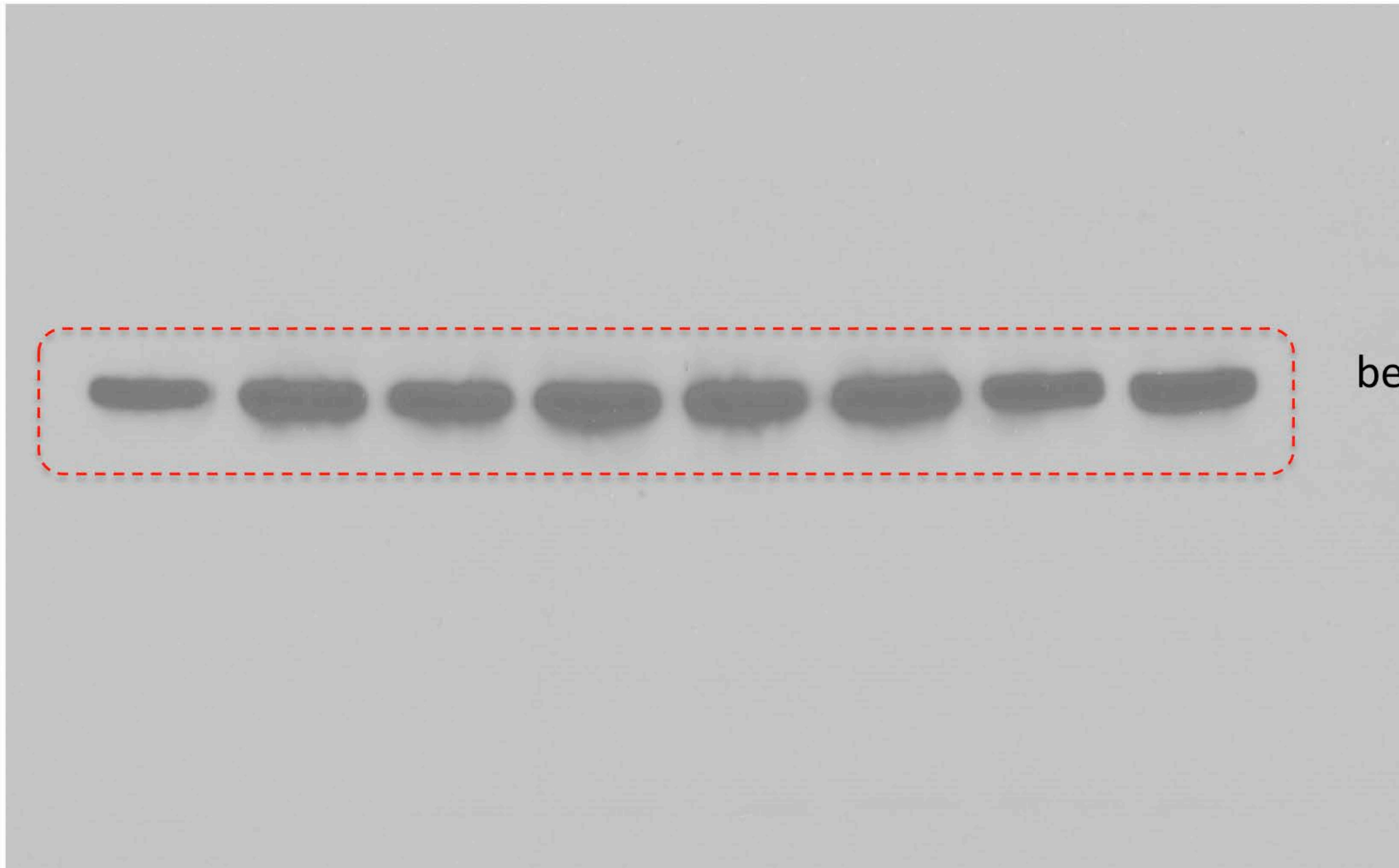
Full unedited gel for Figure 8A-top

A-Cre

A-cKO

Sham TAC

Sham TAC

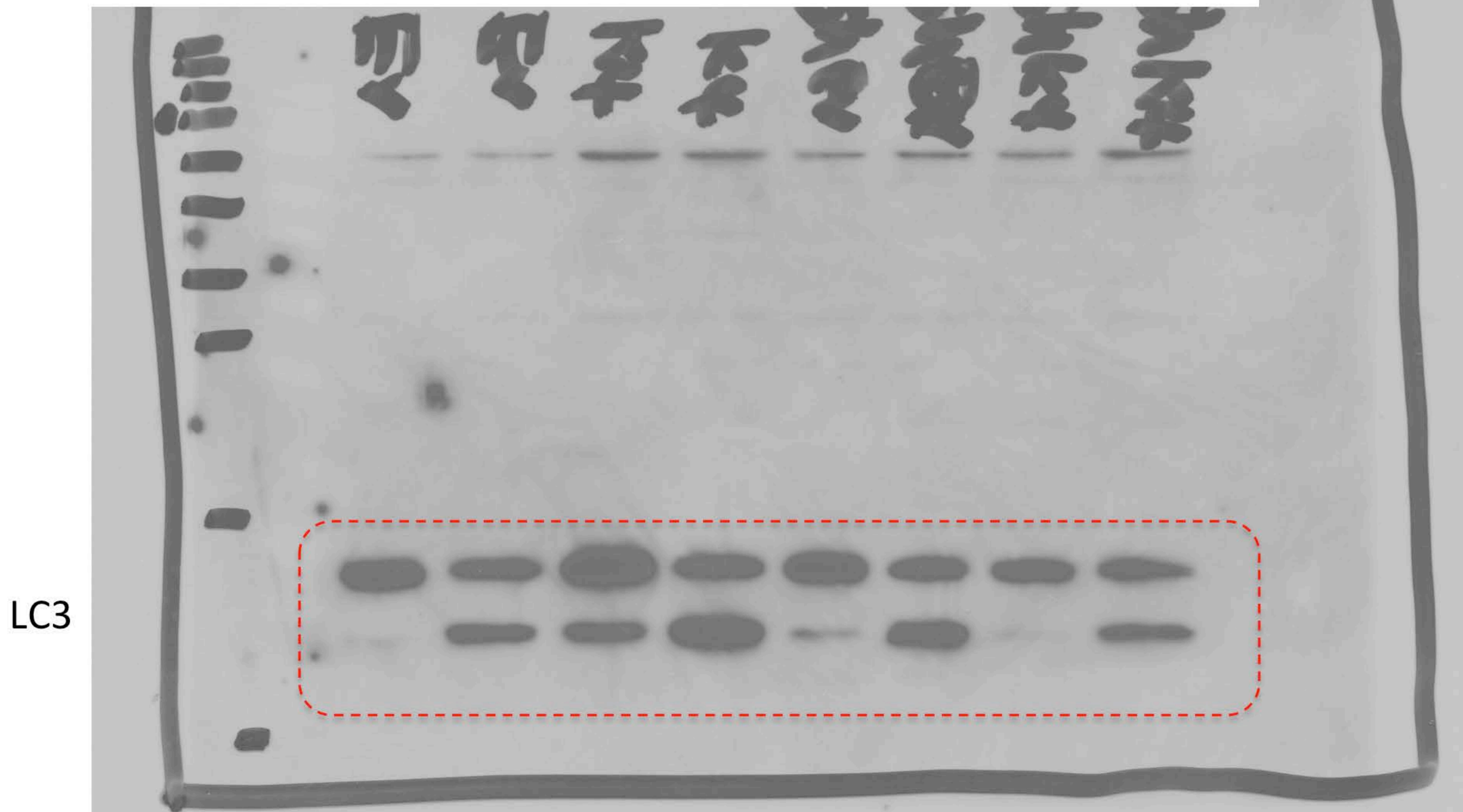


beta-tubulin

Full unedited gel for Figure 8A-middle

NRVM

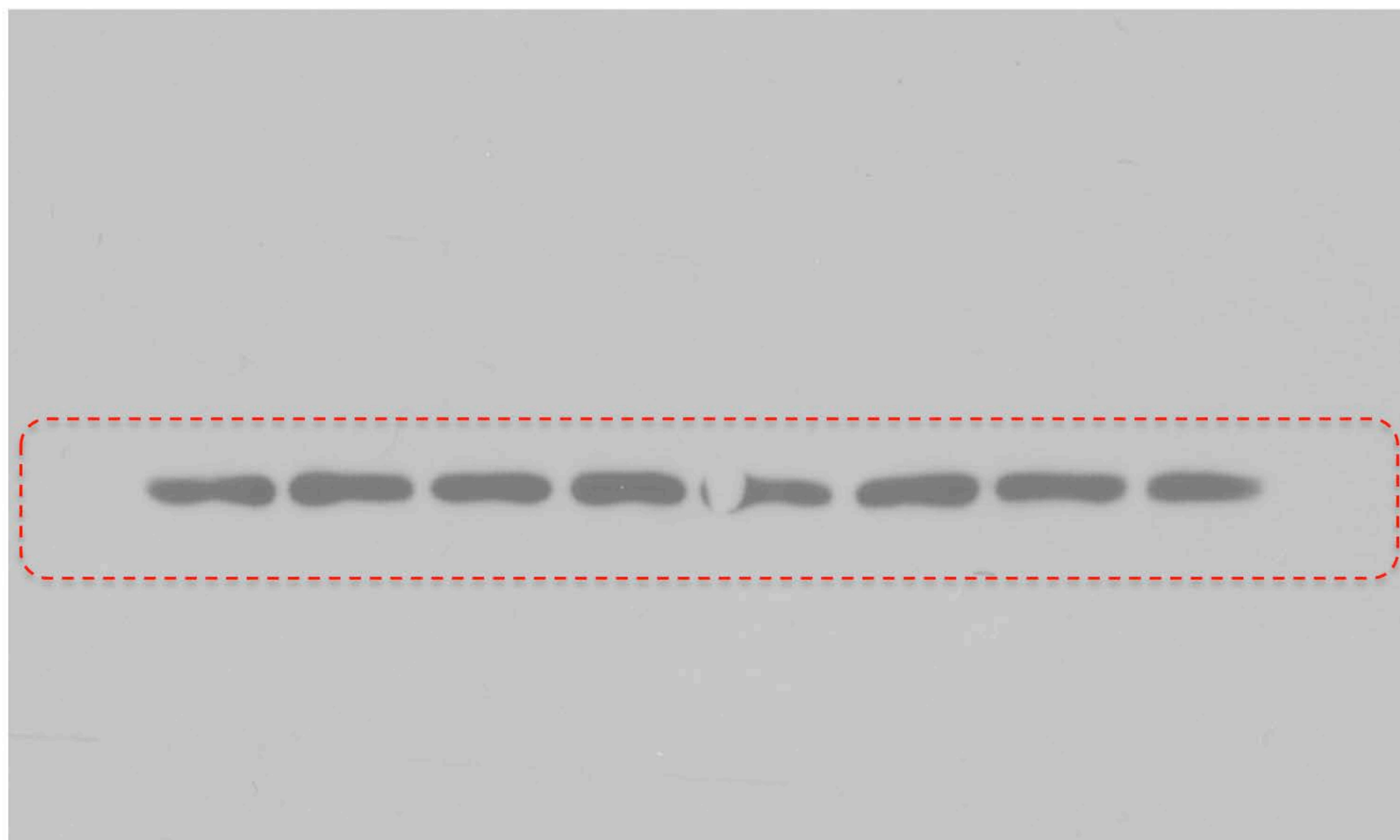
CCCP	-	+	-	+	-	+	-	+
Ad-KLF4	-	-	+	+				
Sh-KLF4					-	-	+	+



Full unedited gel for Figure 8A-middle

NRVM

CCCP	-	+	-	+	-	+	-	+
Ad-KLF4	-	-	+	+				
Sh-KLF4					-	-	+	+

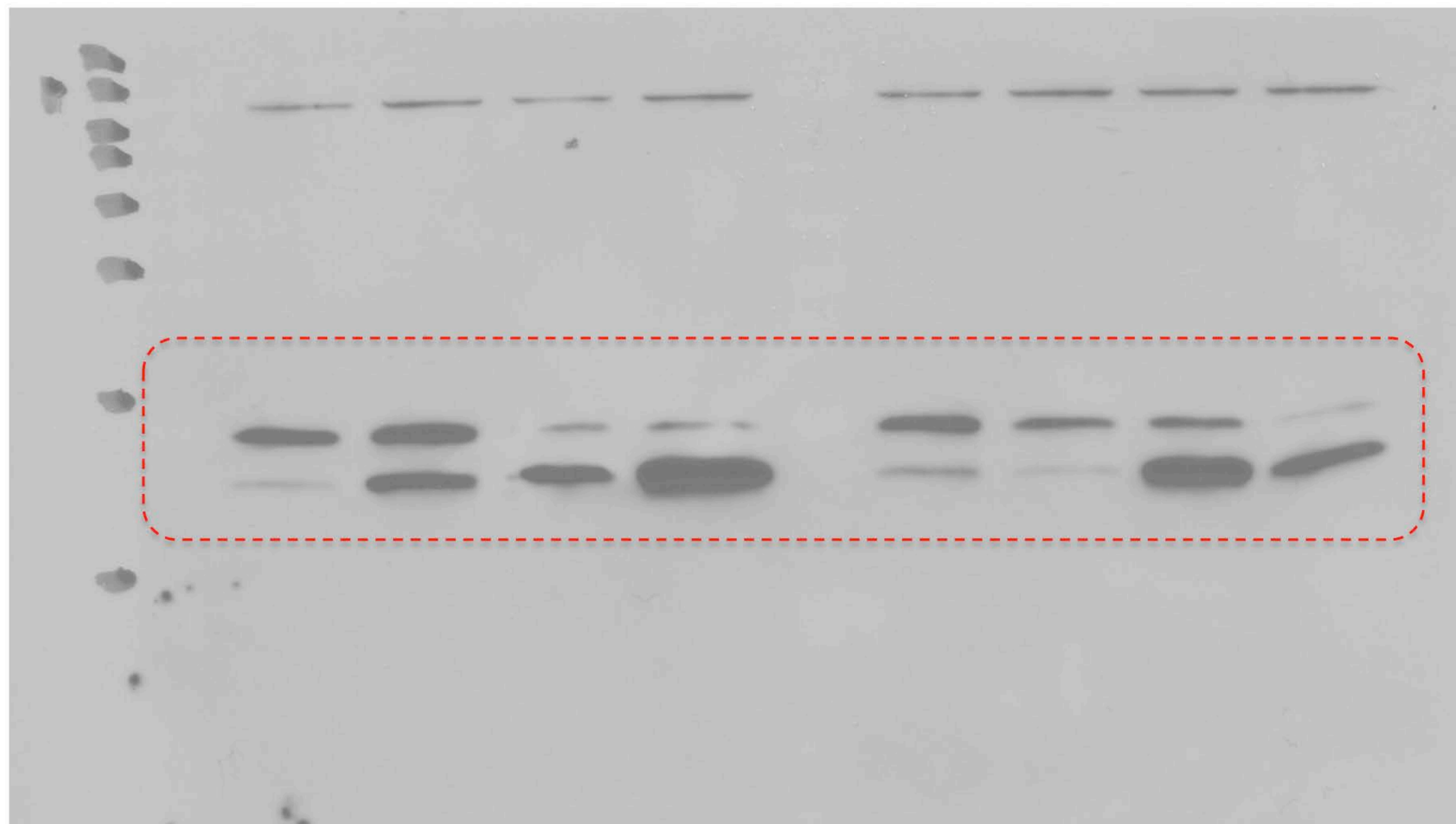


beta-tubulin

Full unedited gel for Figure 8A-bottom

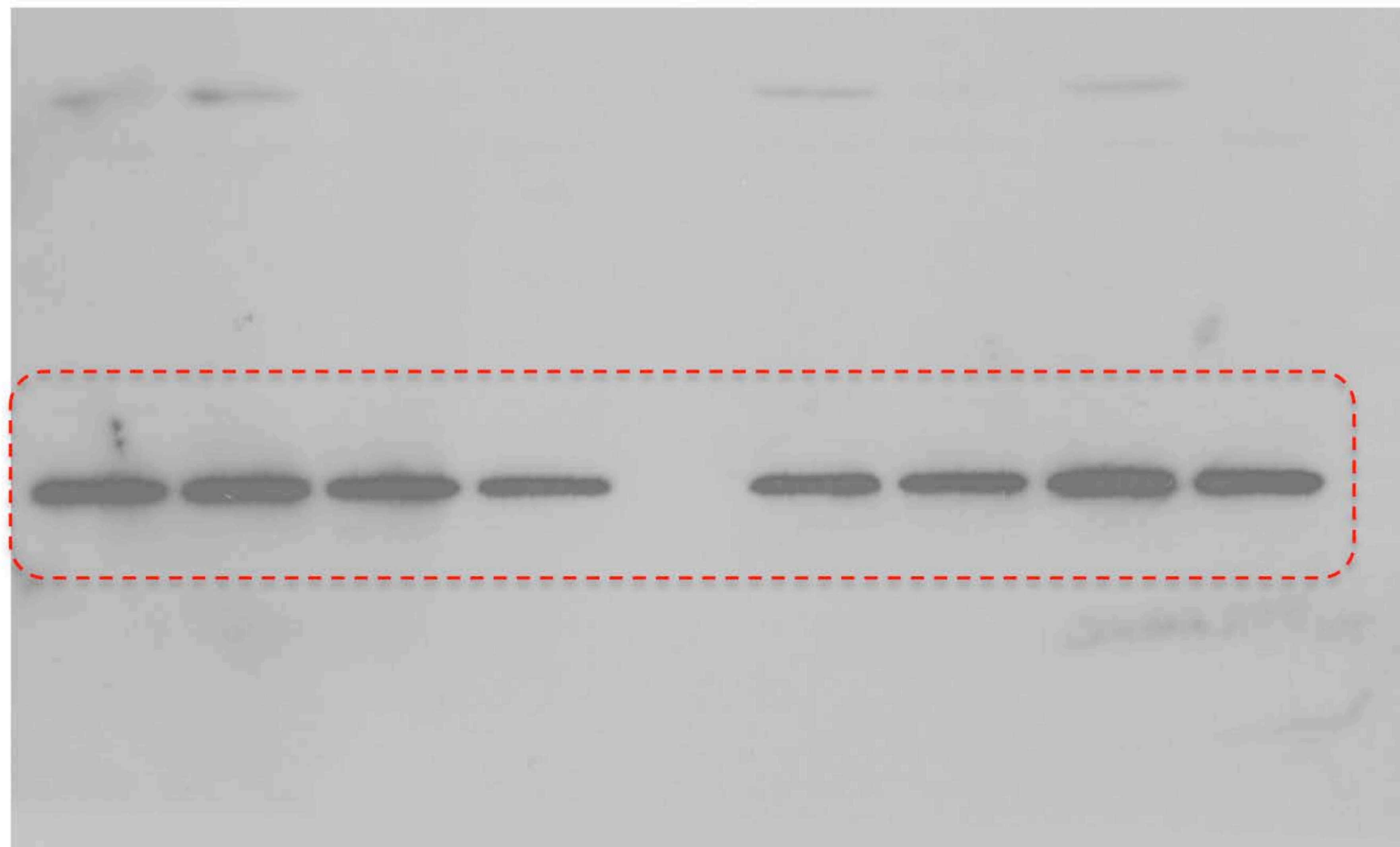
BFA	-	-	+	+	-	-	+	+
Ad-KLF4	-	+	-	+				
Sh-KLF4					-	+	-	+

LC3



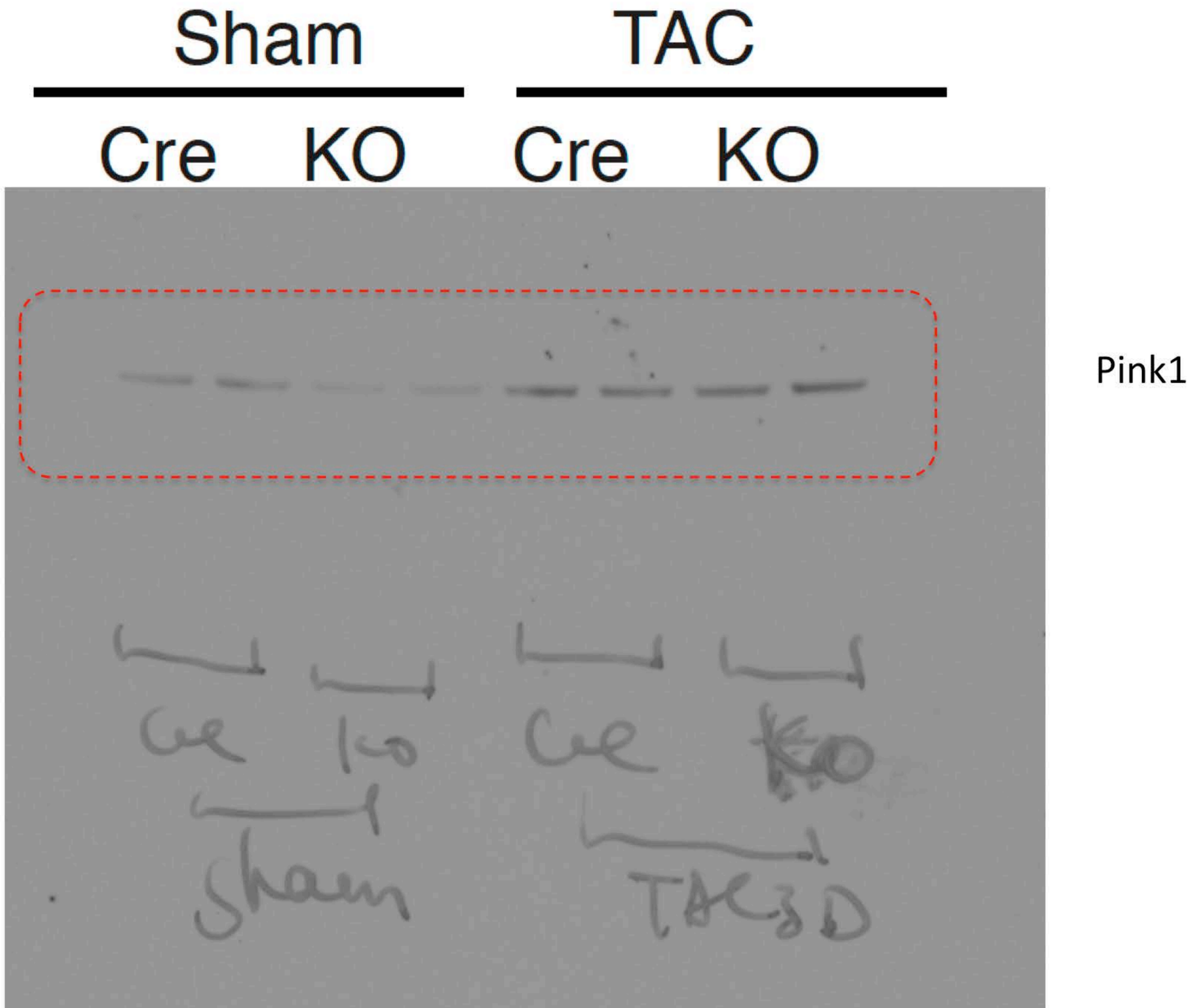
Full unedited gel for Figure 8A-bottom

BFA	-	-	+	+	-	-	+	+
Ad-KLF4	-	+	-	+				
Sh-KLF4					-	+	-	+



beta-tubulin

Full unedited gel for Figure 8B-top



Full unedited gel for Figure 8B-top

Sham

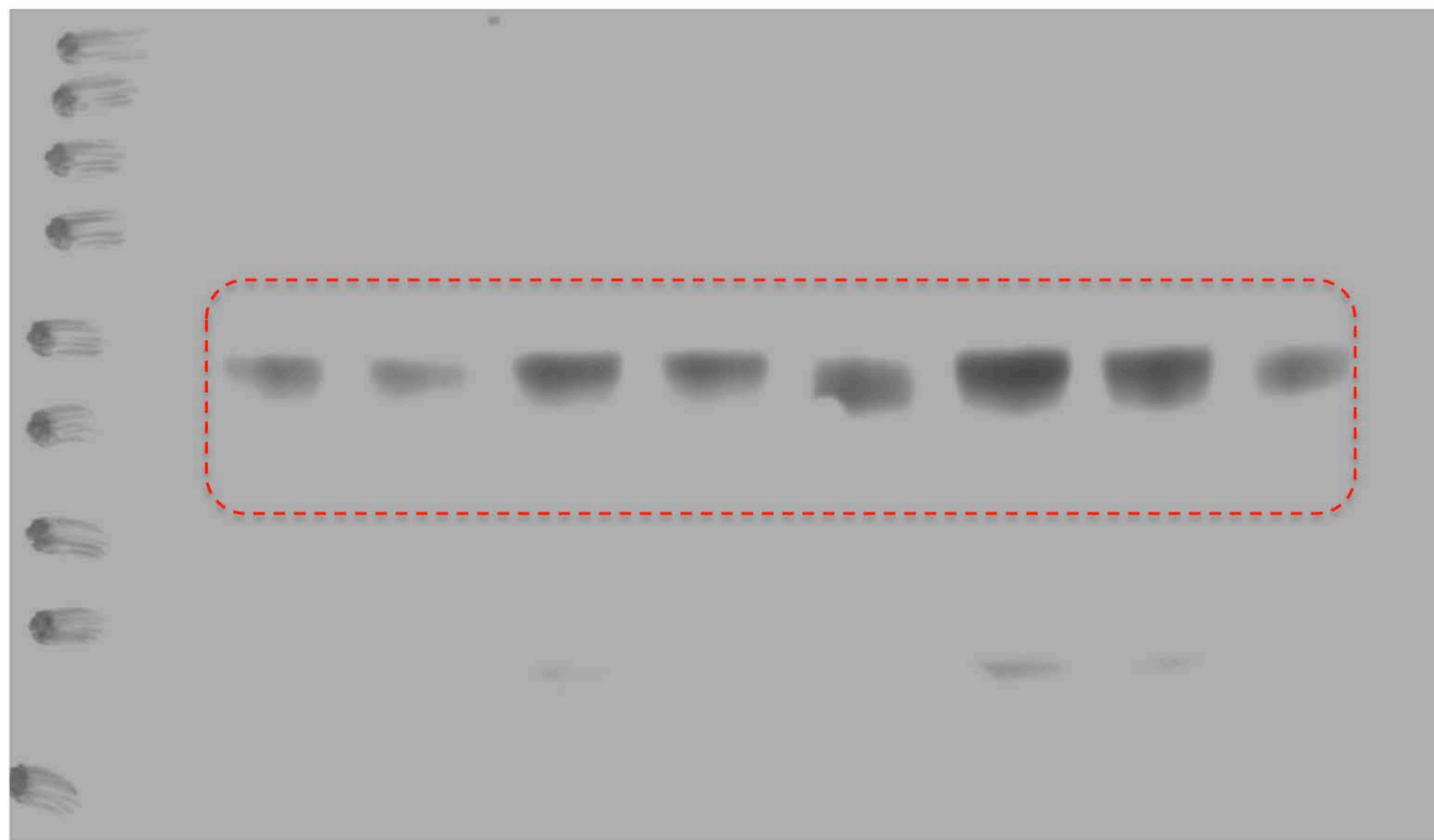
TAC

Cre

KO

Cre

KO



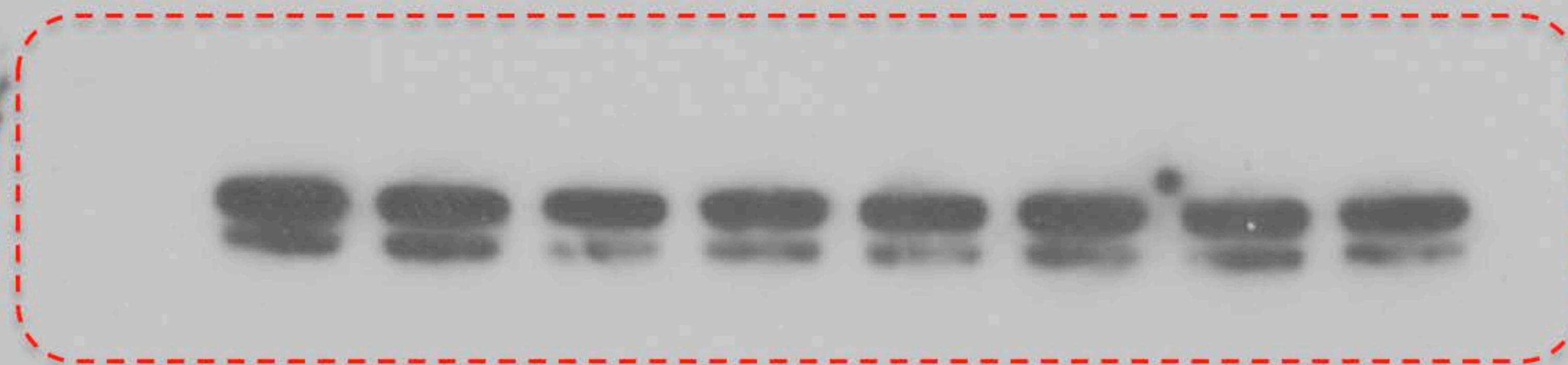
Parkin

Full unedited gel for Figure 8B-top

Sham		TAC	
Cre	KO	Cre	KO

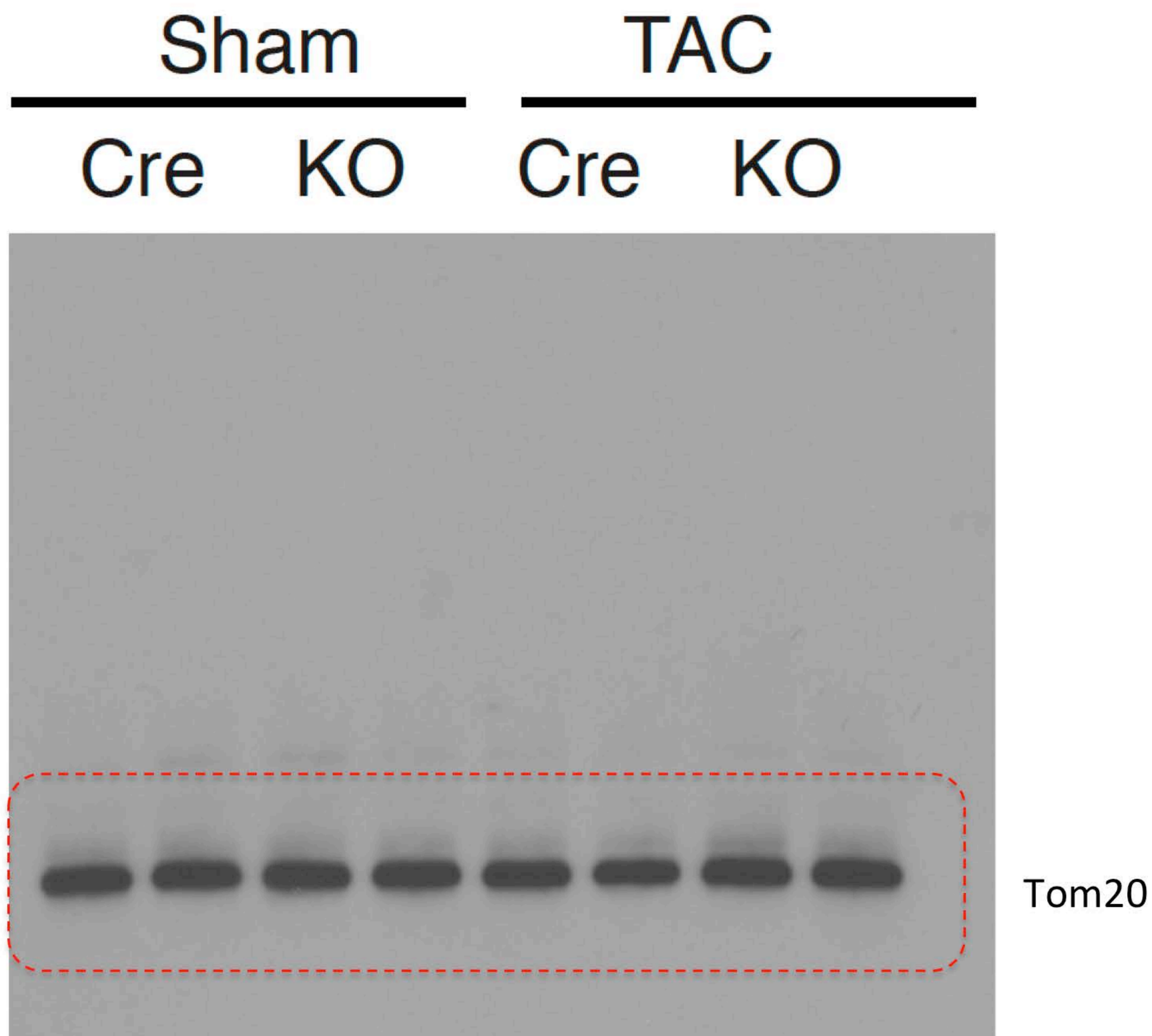
Mito

p62



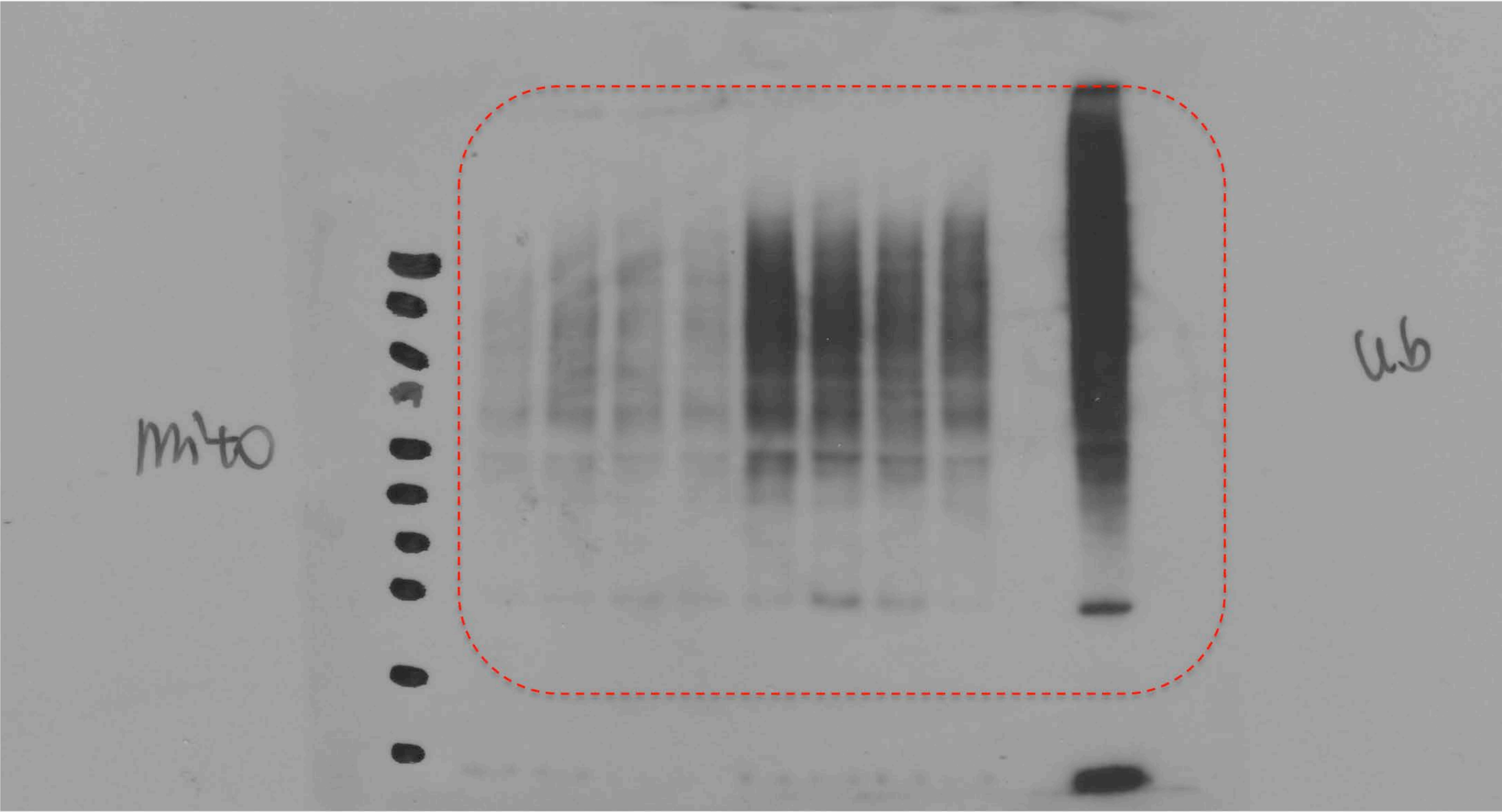
P62

Full unedited gel for Figure 8B-top

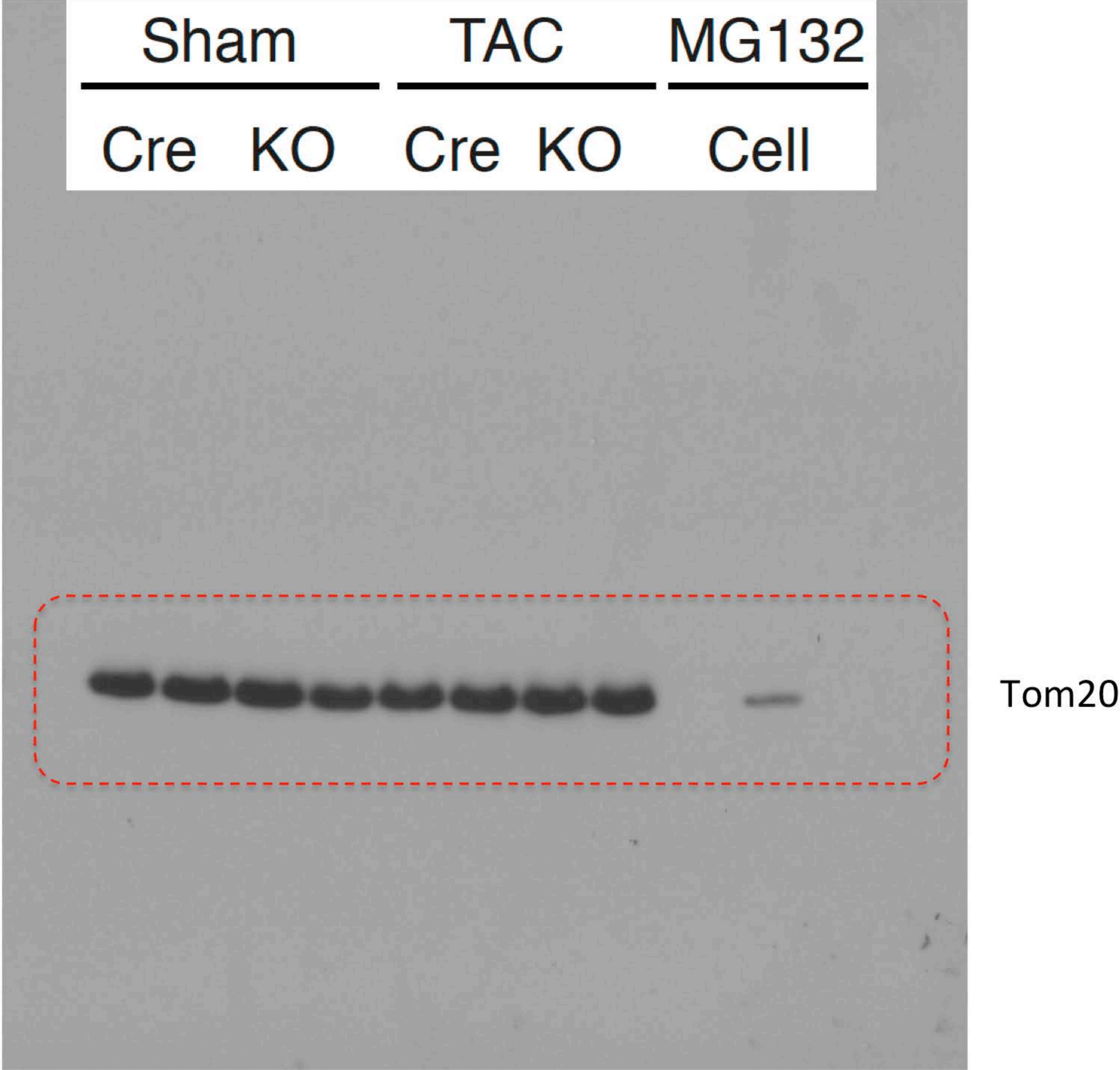


Full unedited gel for Figure 8B-bottom

Sham		TAC		MG132
Cre	KO	Cre	KO	Cell



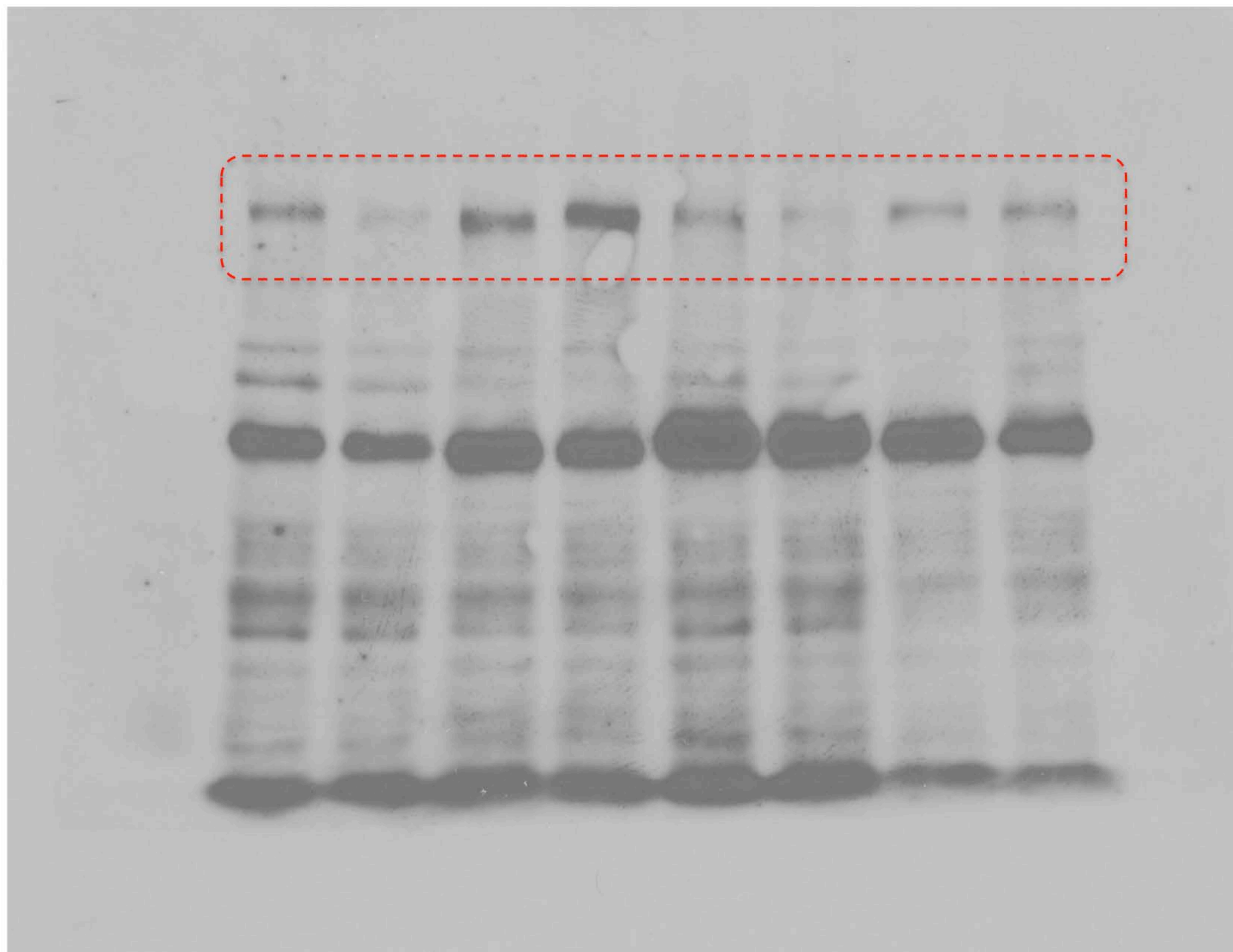
Full unedited gel for Figure 8B-bottom



Full unedited gel for Figure 8C-top

A-Cre **A-cKO**

Sham **TAC** **Sham** **TAC**



p-ULK1

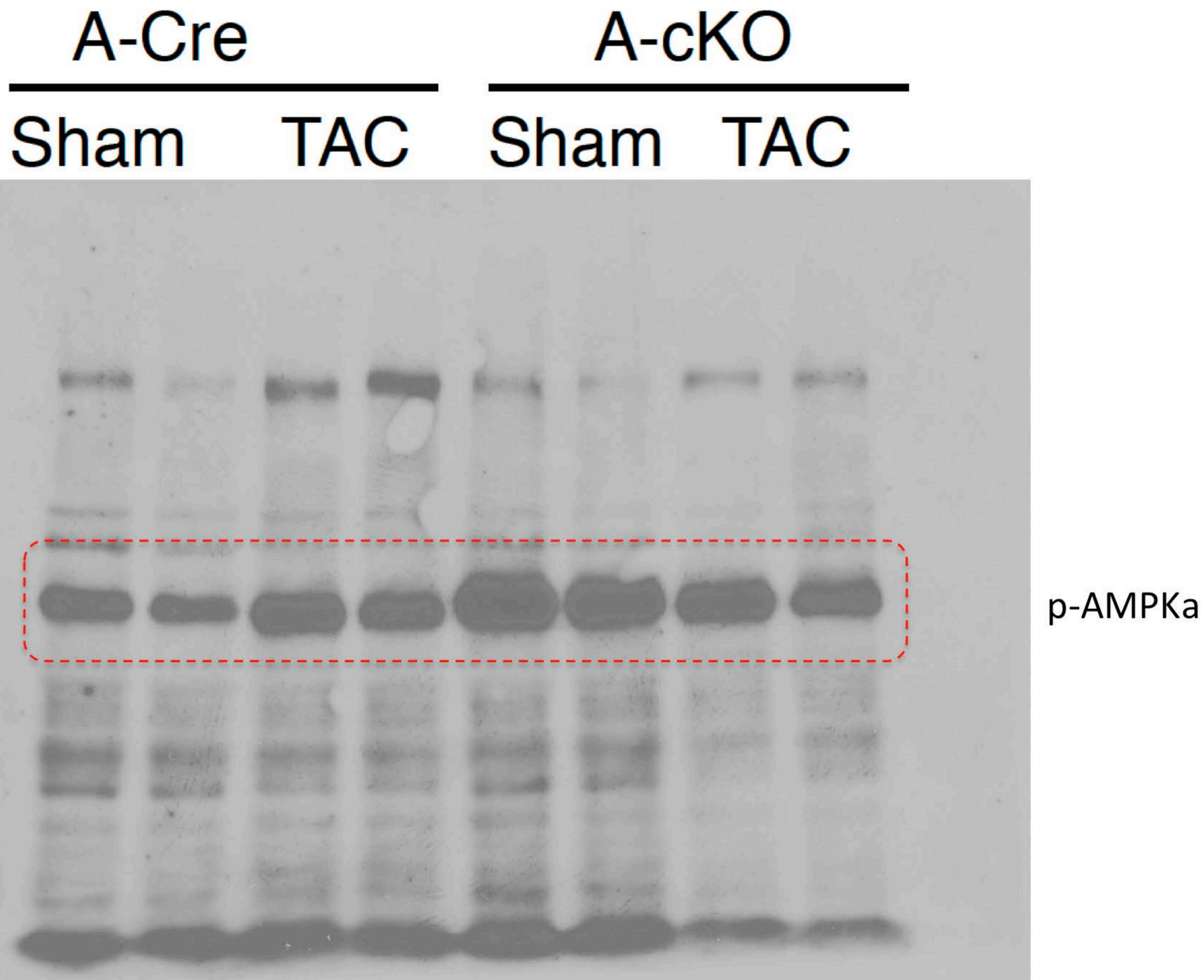
Full unedited gel for Figure 8C-top

A-Cre A-cKO
Sham TAC Sham TAC

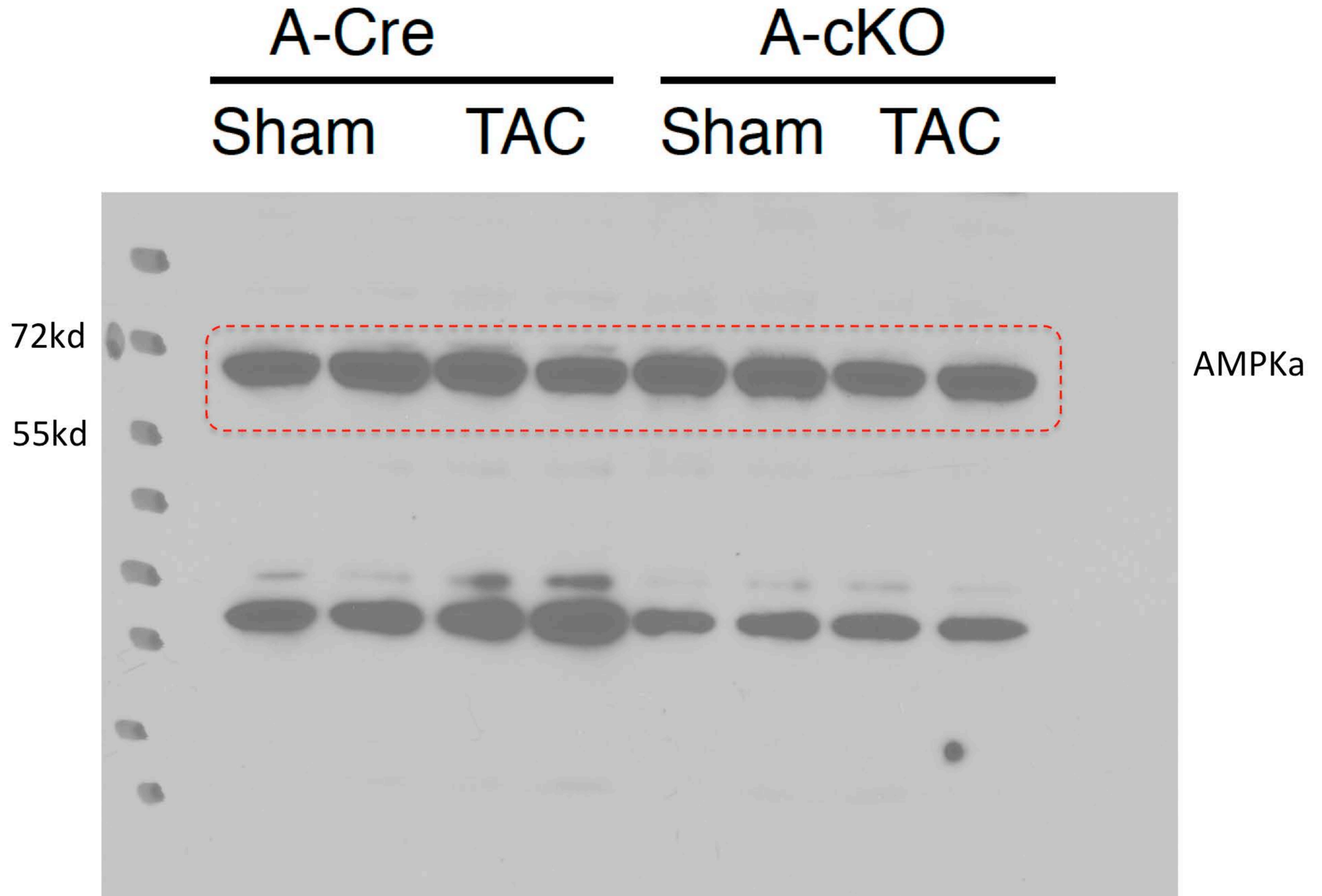


ULK1

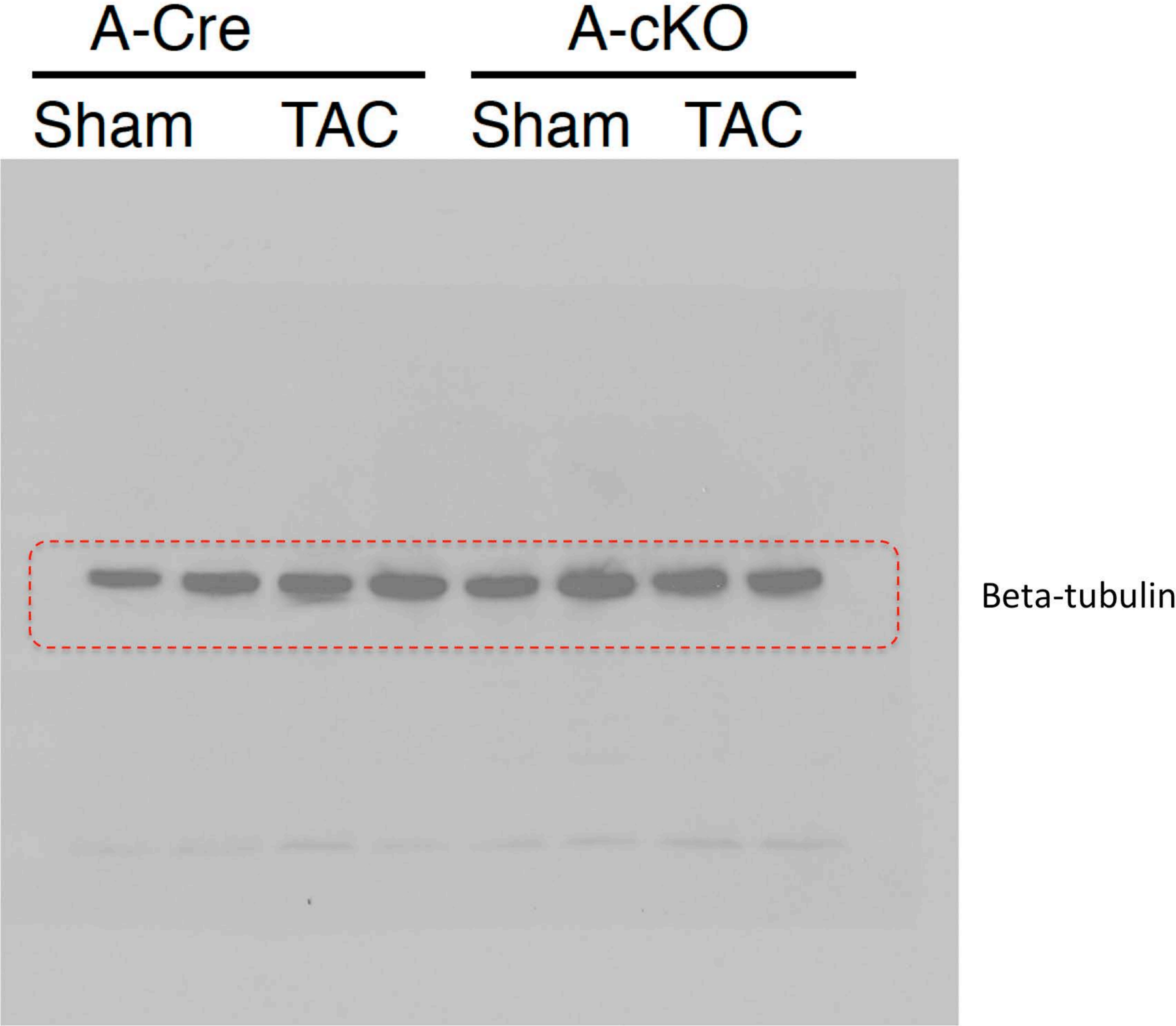
Full unedited gel for Figure 8C-top



Full unedited gel for Figure 8C-top

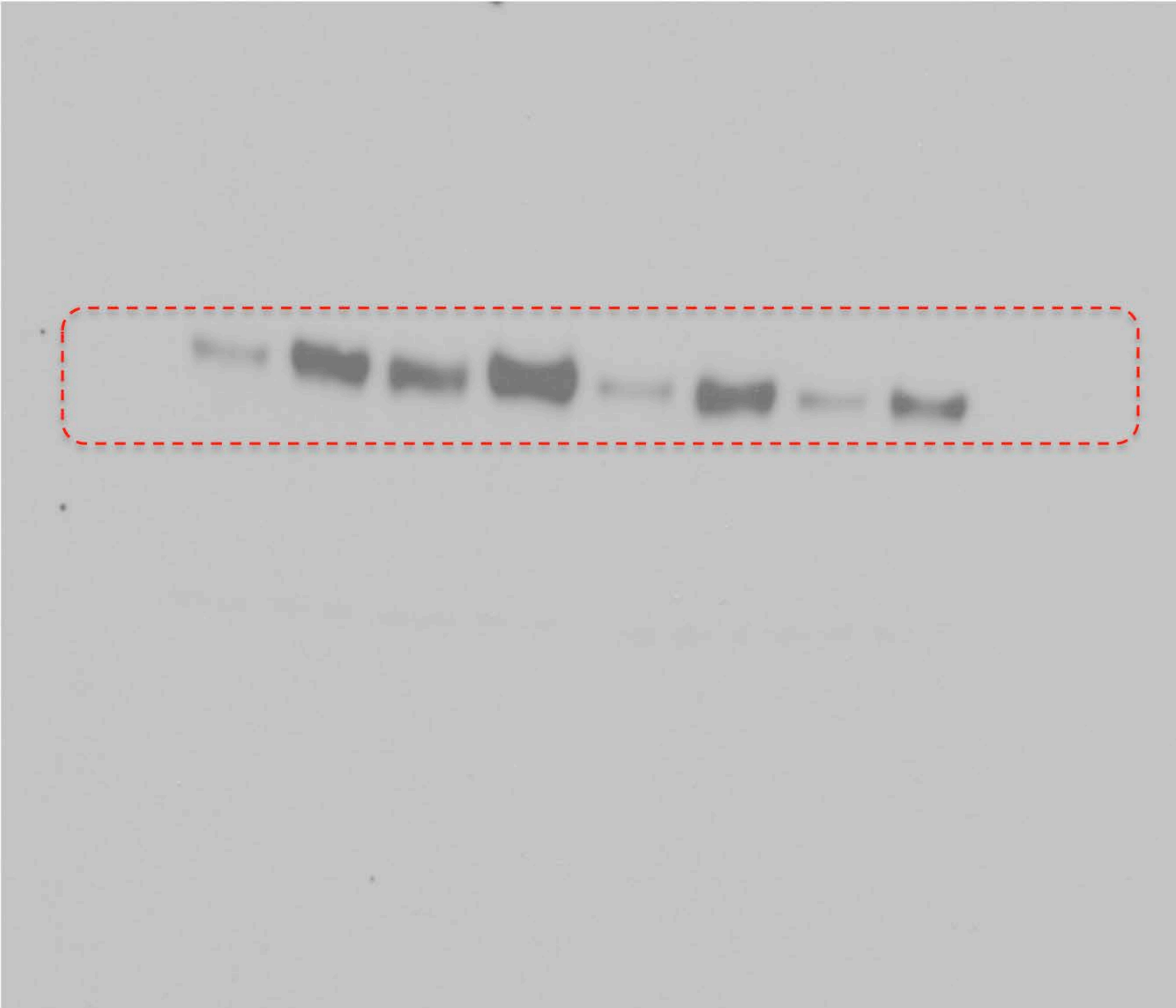


Full unedited gel for Figure 8C-top



Full unedited gel for Figure 8C-bottom

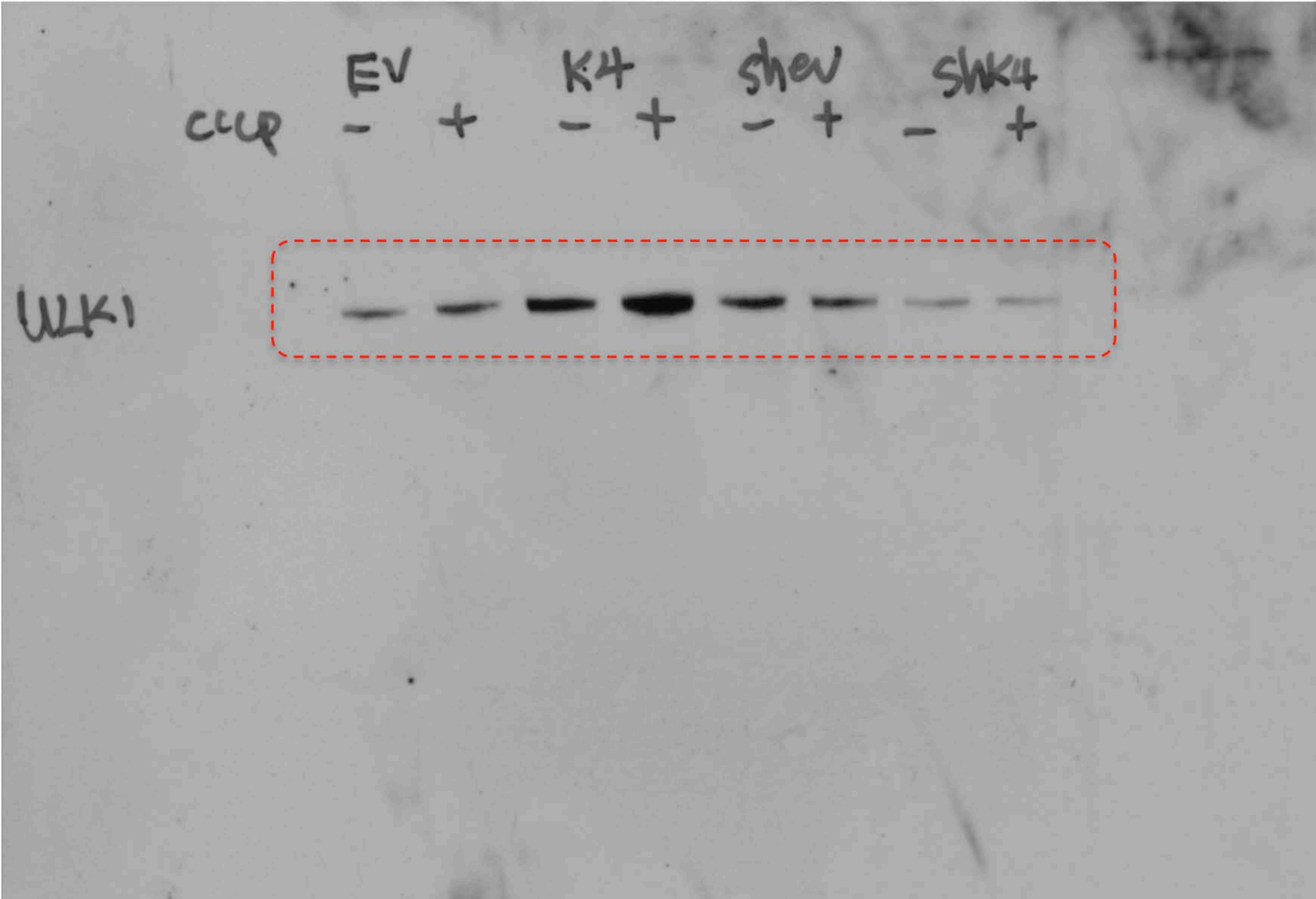
CCCP	-	+	-	+	-	+	-	+
Ad-KLF4	-	-	+	+				
Sh-KLF4					-	-	+	+



p-ULK1

Full unedited gel for Figure 8C-bottom

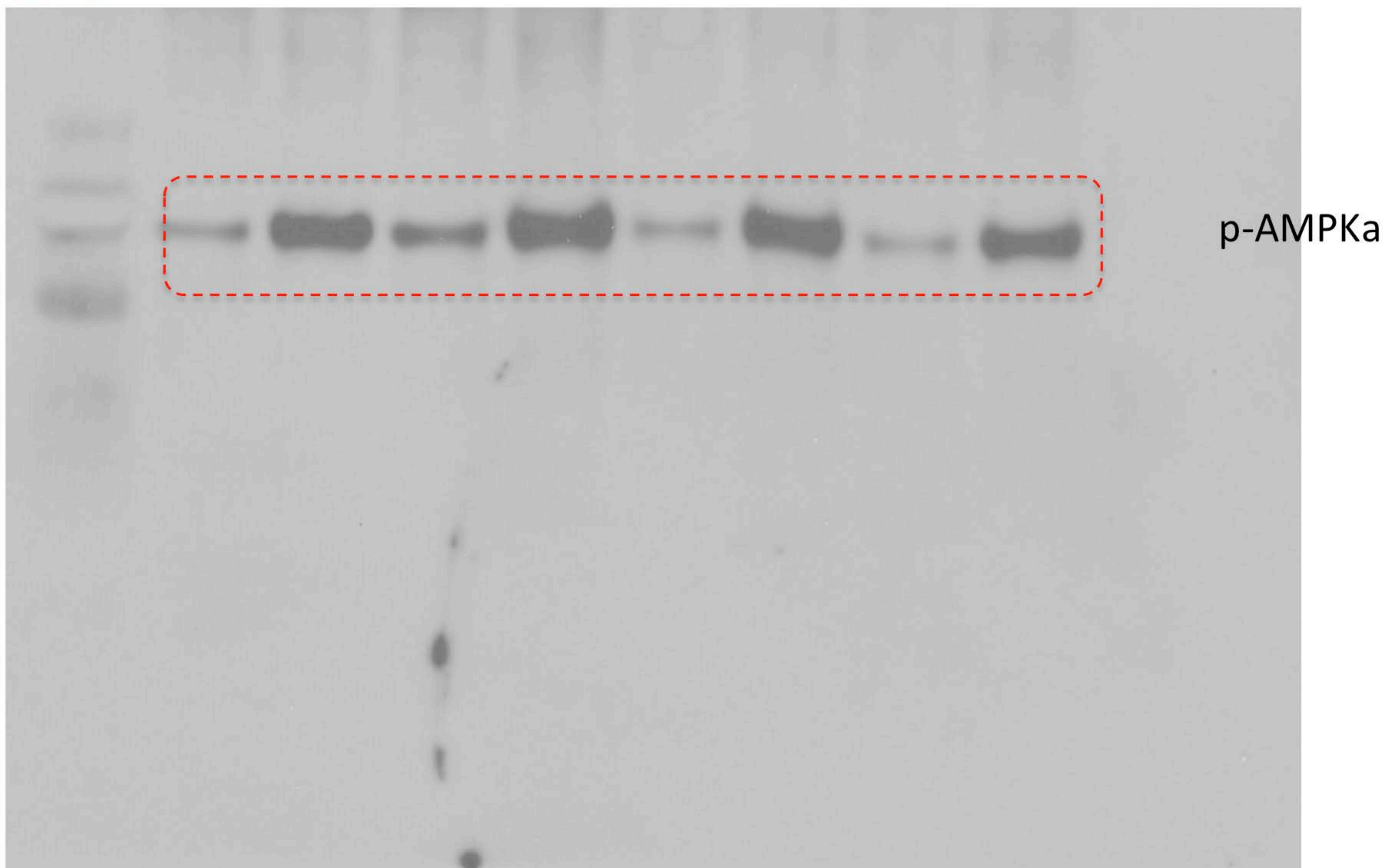
CCCP	-	+	-	+	-	+	-	+
Ad-KLF4	-	-	+	+				
Sh-KLF4					-	-	+	+



ULK1

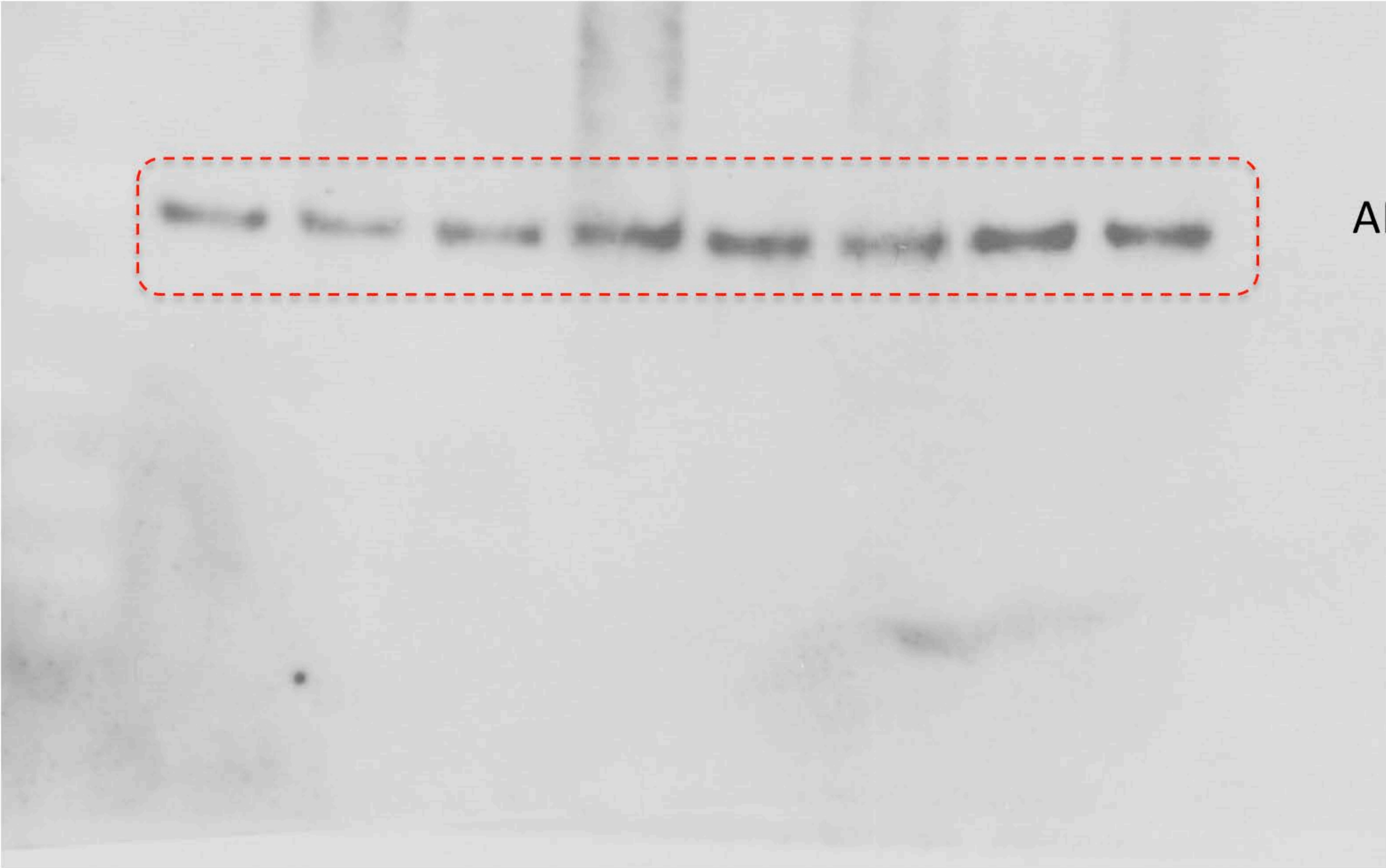
Full unedited gel for Figure 8C

CCCP	-	+	-	+	-	+	-	+
Ad-KLF4	-	-	+	+				
Sh-KLF4					-	-	+	+



Full unedited gel for Figure 8C

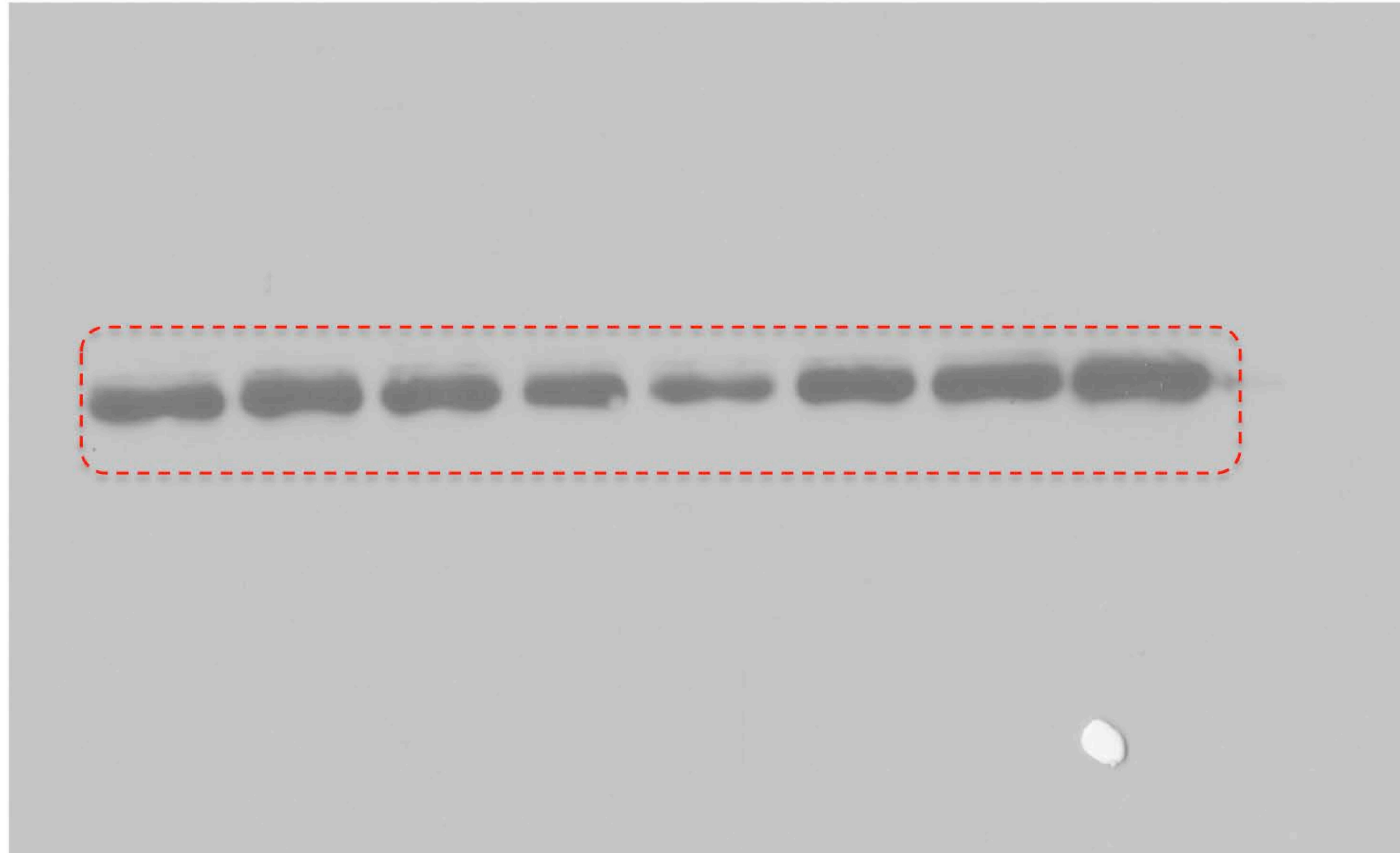
CCCP	-	+	-	+	-	+	-	+
Ad-KLF4	-	-	+	+				
Sh-KLF4					-	-	+	+



AMPKα

Full unedited gel for Figure 8C

CCCP	-	+	-	+	-	+	-	+
Ad-KLF4	-	-	+	+				
Sh-KLF4					-	-	+	+

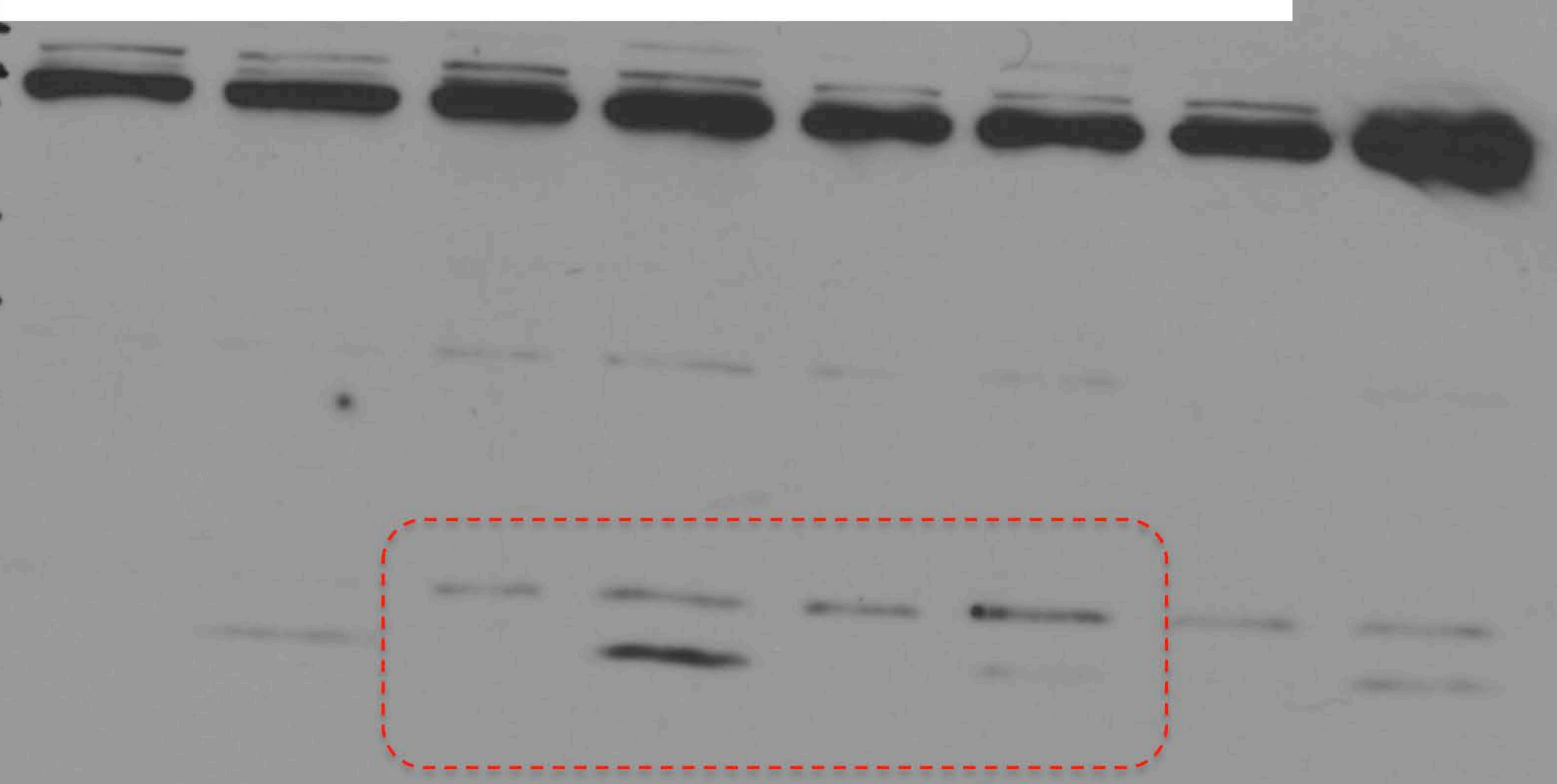


Beta-tubulin

Full unedited gel for

NRVM

	Sh-EV		Sh-ULK1	
Ad-EV	+	-	+	-
Ad-KLF4	-	+	-	+



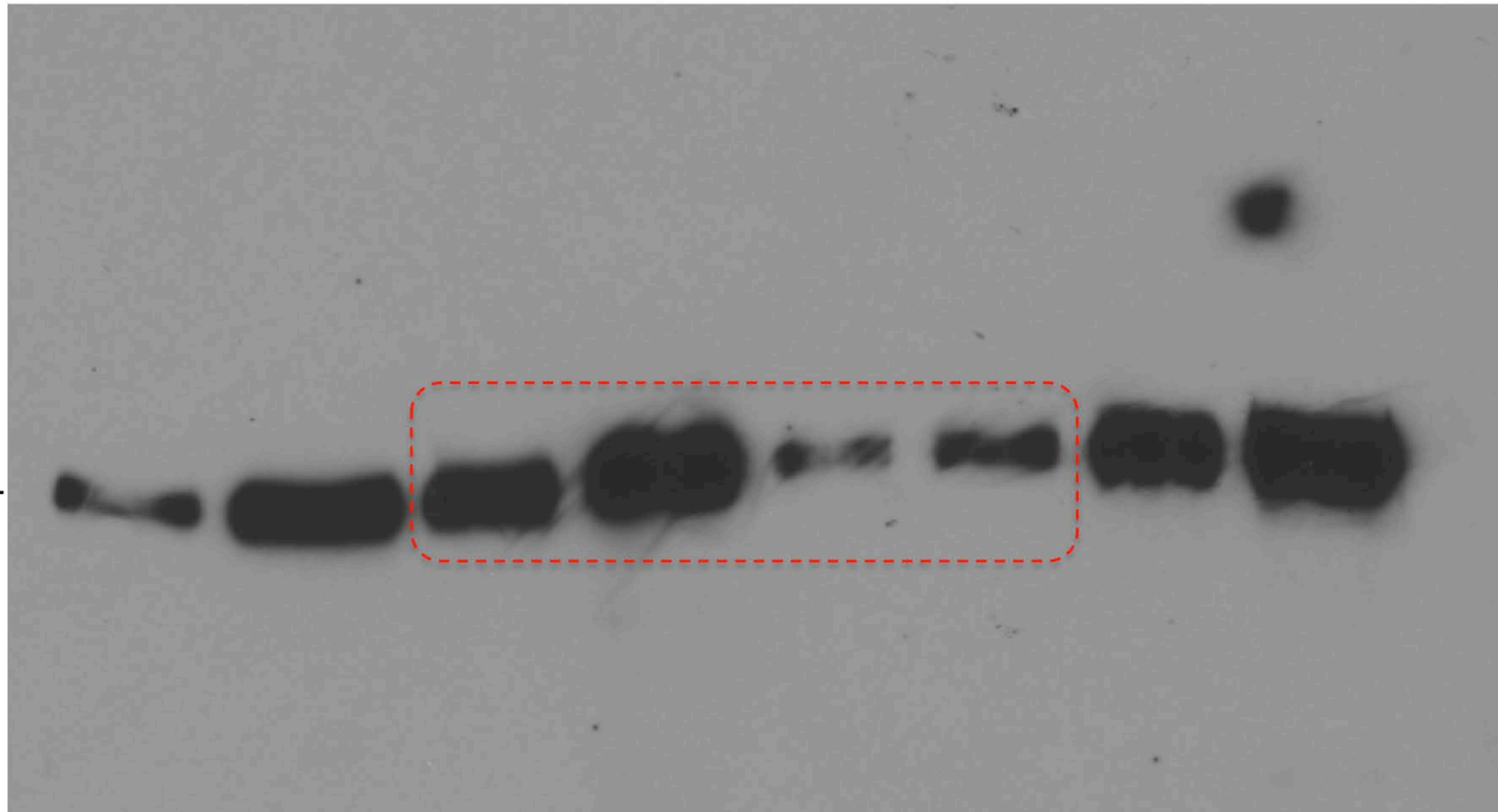
143

Full unedited gel for Figure 8D

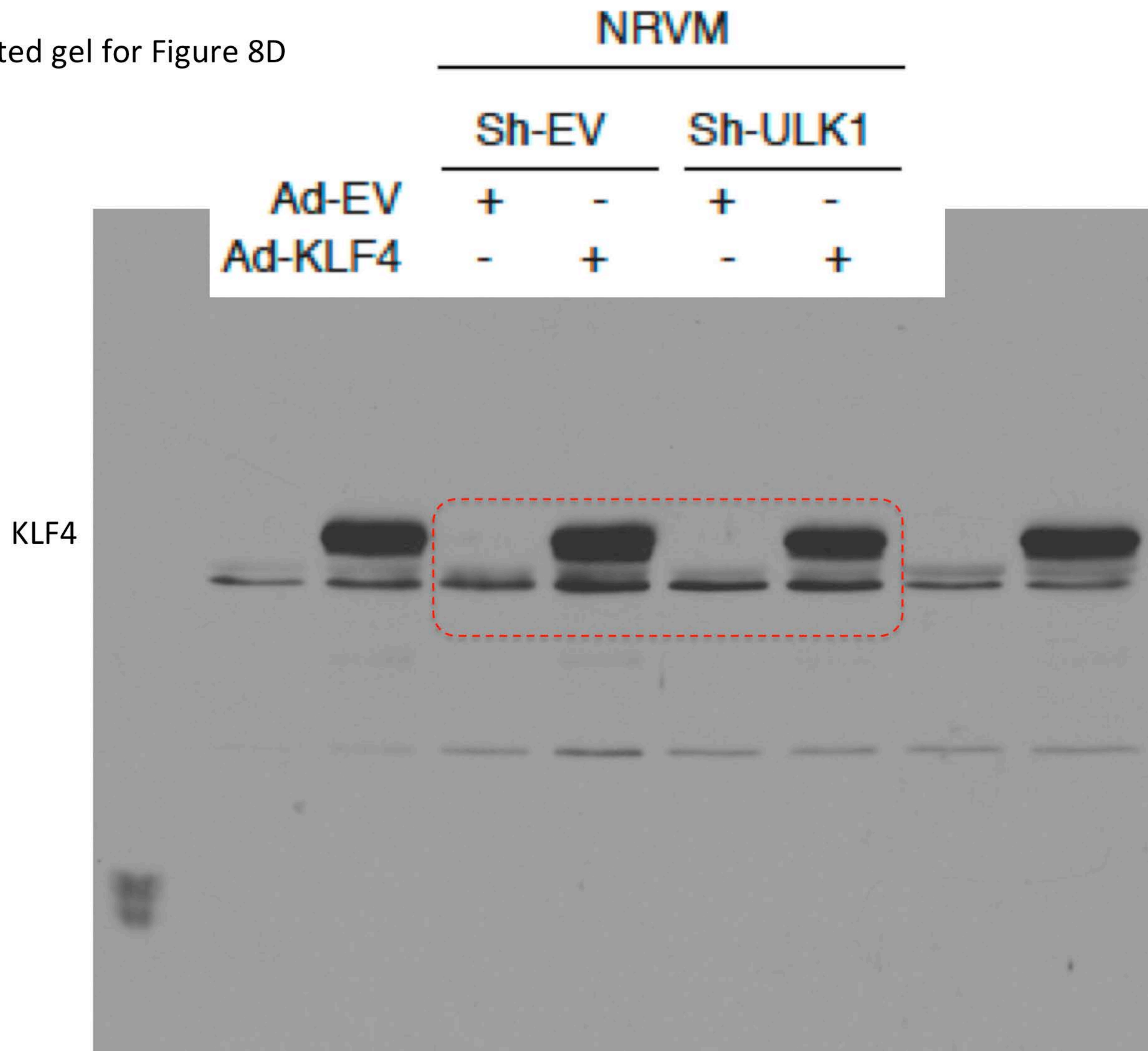
NRVM

	Sh-EV		Sh-ULK1	
Ad-EV	+	-	+	-
Ad-KLF4	-	+	-	+

ULK1



Full unedited gel for Figure 8D

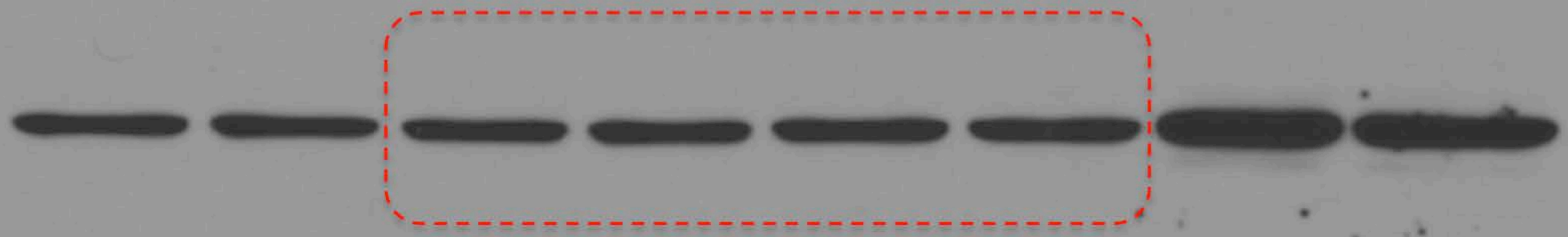


Full unedited gel for Figure 8D

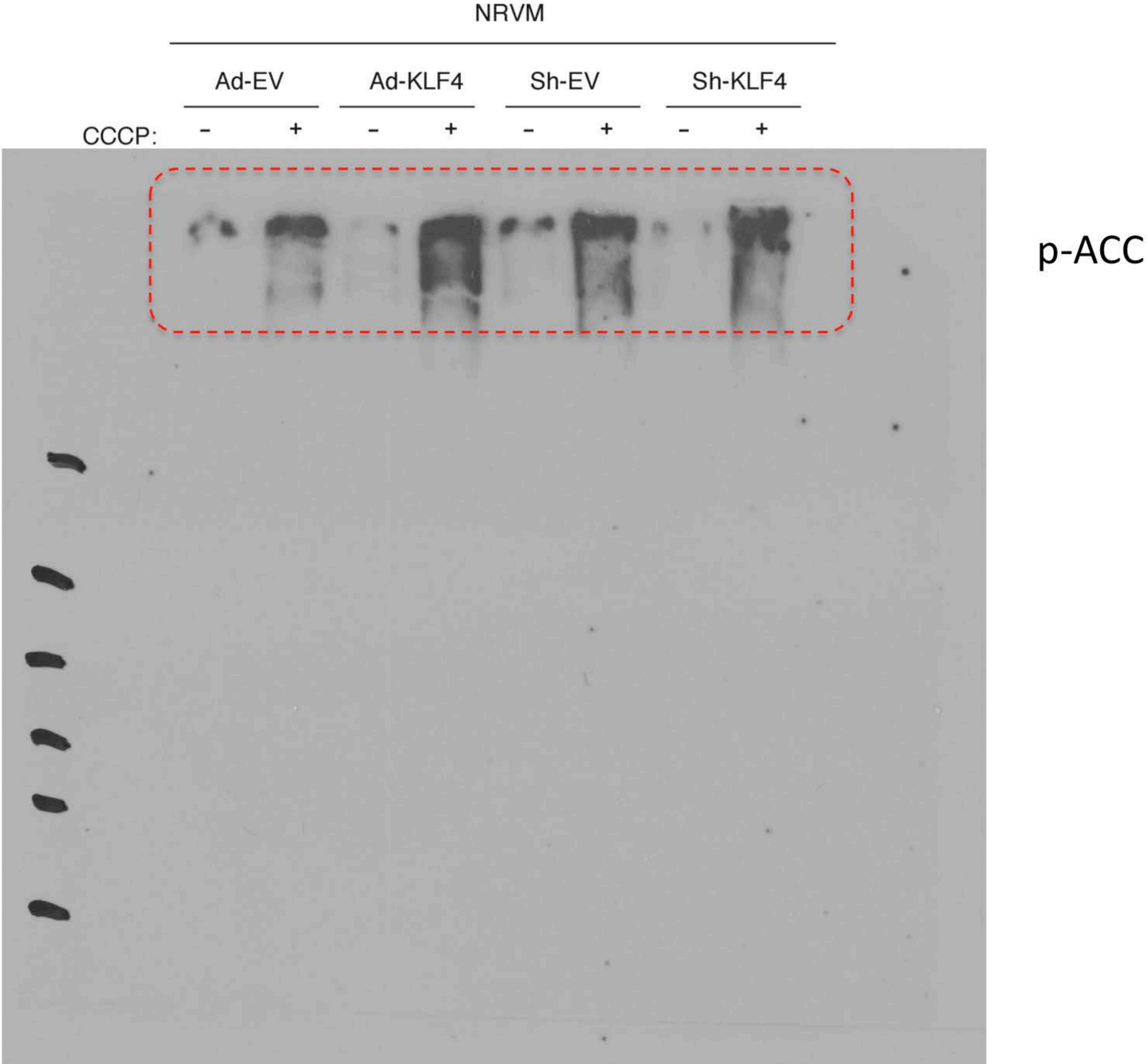
NRVM

	NRVM			
	Sh-EV		Sh-ULK1	
Ad-EV	+	-	+	-
Ad-KLF4	-	+	-	+

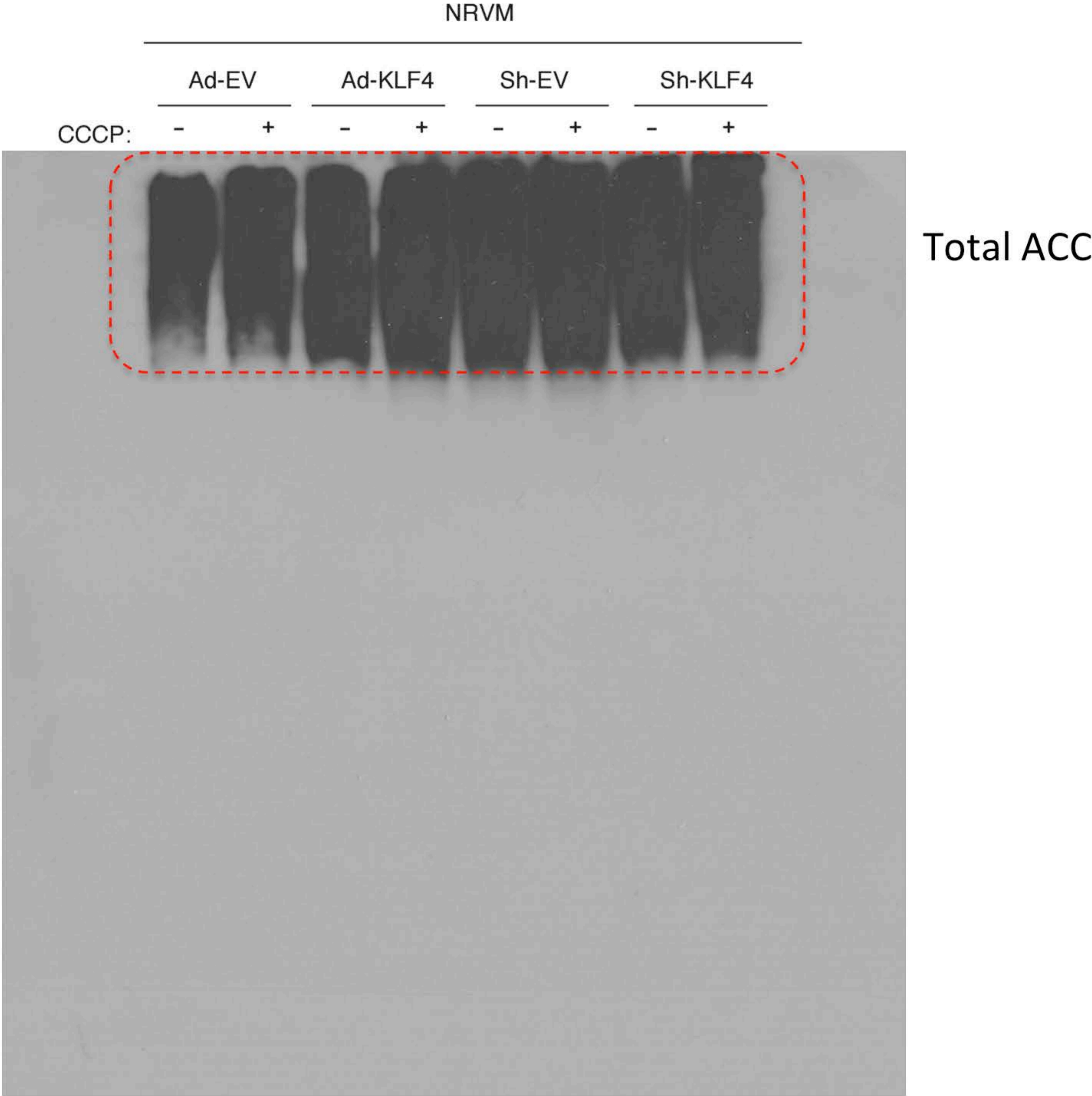
beta-tubulin



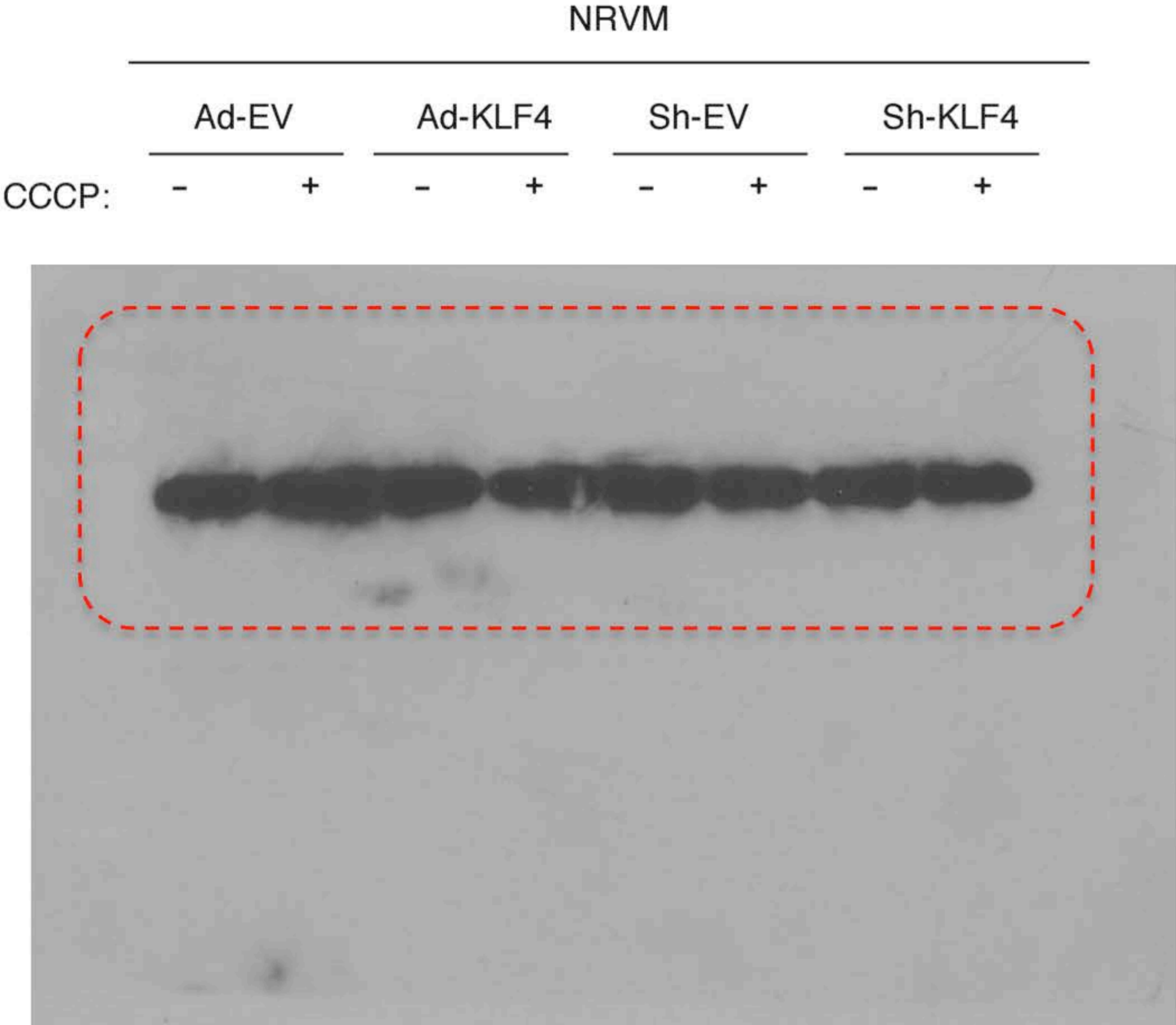
Full unedited gel for Supplementary Figure 12



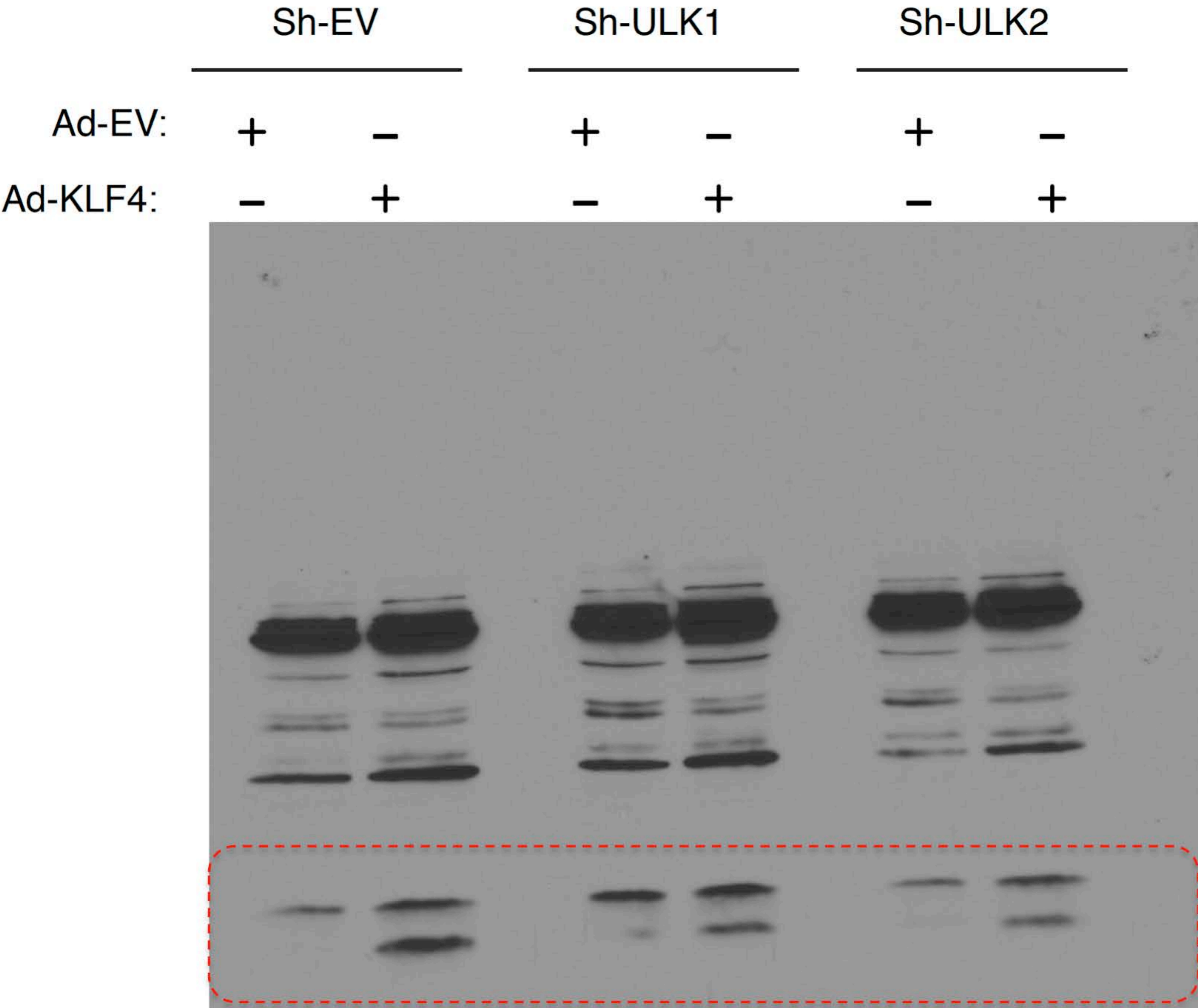
Full unedited gel for Supplementary Figure 12



Full unedited gel for Supplementary Figure 12



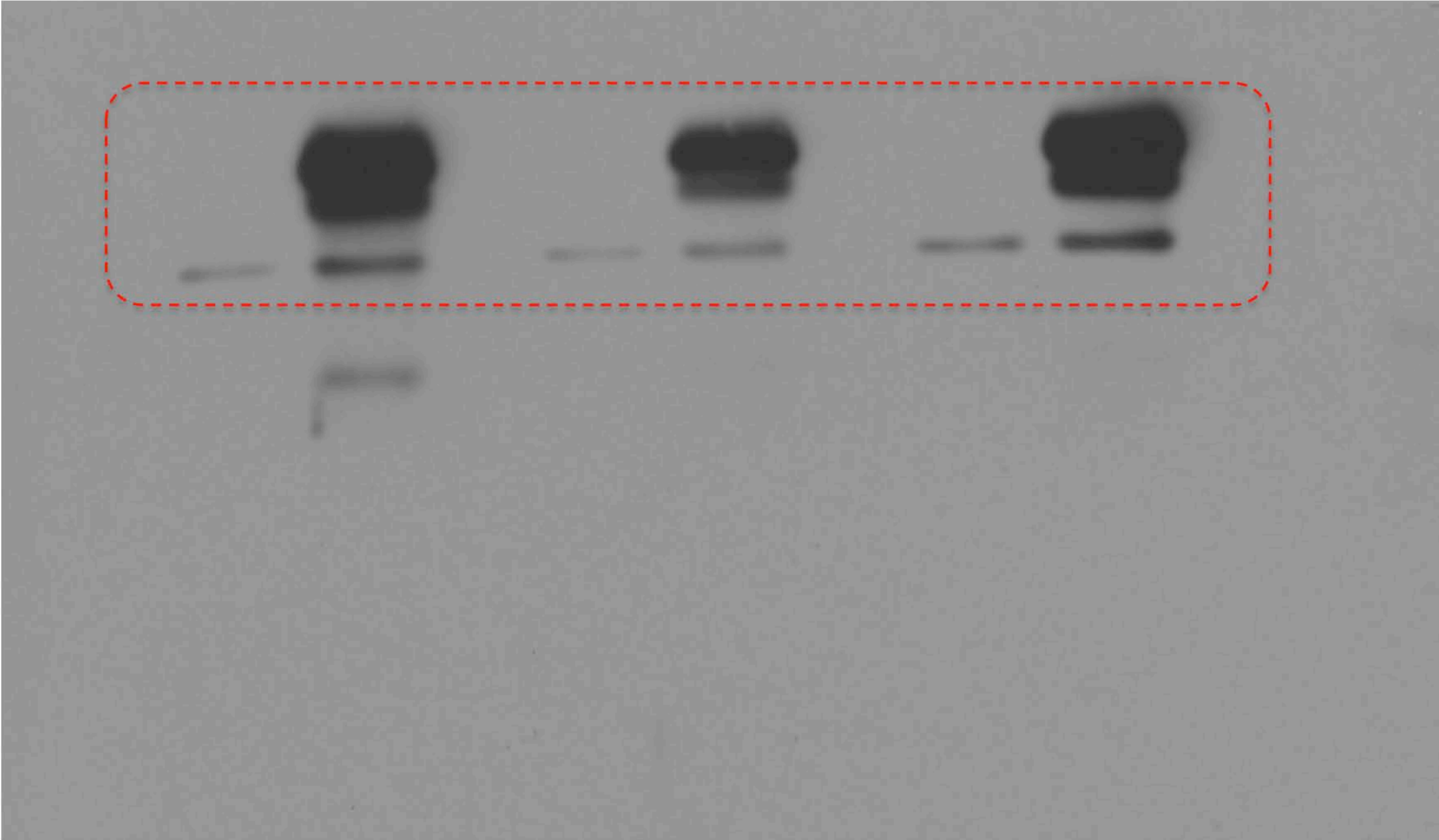
Full unedited gel for Supplementary Figure 14D



LC3

Full unedited gel for Supplementary Figure 14D

	Sh-EV		Sh-ULK1		Sh-ULK2	
Ad-EV:	+	-	+	-	+	-
Ad-KLF4:	-	+	-	+	-	+



KLF4

Full unedited gel for Supplementary Figure 14D

