Supplementary Data S1

TRPV4 acetylation in prenatal liver prevents low glucose-induced inhibition of mTORC1 and safeguards fetal development

This file includes:

Full sequence of TRPV4-WT, TRPV4-K608R, GCaMP6s

The details of constructing gene knock-in mouse models

Rosa26-LSL-TRPV4-WT-HA Genotyping Protocol

Rosa26-LSL-TRPV4-K608R-HA Genotyping Protocol

Gel source data

Full sequence: TRPV4-WT Homo sapiens CCDS9134.1

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Full sequence: TRPV4-K608R Homo sapiens

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Full sequence: GCaMP6s

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SMOC-Report for Rosa26 knock-in mouse model (TRPV4-WT)

Contents

1. Background and Objective

1.1 Objective

Generation of the CAG-LSL-TRPV4-WT-HA-WPRE-pA cassette knock-in mouse model at Rosa26 gene locus via CRISPR/Cas9 technology.

1.2 Background

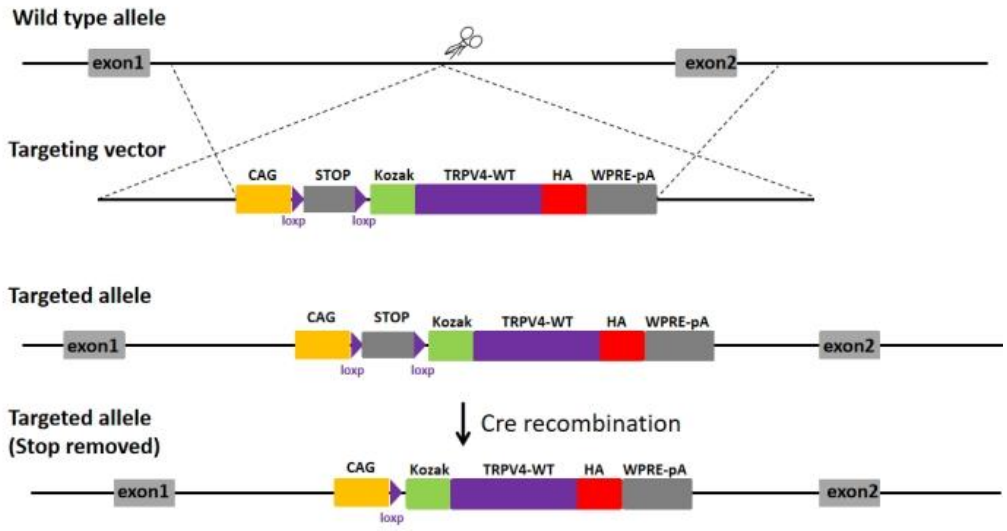
Gene Name (MGI Number): Gt(ROSA)26Sor (104735)
Gene URL Link (MGI) : http://www.informatics.jax.org/marker/MGI:104735
Gene URL Link (Ensembl) : http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000086429;r=6:113067428-113077333
Knockin Cassette: CAG-LSL-TRPV4-WT-HA-WPRE-pA

2. Abstract

The project process was as follows: 1) Cas9 mRNA and gRNA were produced by in vitro transcription; 2) donor vectors were constructed by in-fusion. The plasmid structure contains 5'homologous arm (3.3 kb), Knock-in, 3'homologous arm (3.3 kb) ; 3) the mixture of Cas9 mRNA,gRNA and donor vector was micro-injected into fertilized eggs (C57BL/6J), then 1 F0 mouse that identified by PCR and sequencing was generated; 4) F0 positive mouse was crossed with wild-type C57BL/6J mice to generate 6 F1 mice.

3. Recombinant strategy

3.1 Strategy figure：



Strategy figure

3.2 Sequence of sgRNAs

gRNAs	Sequence (5'-3')
gRNA1	GGGGACACACTAAGGGAGCT TGG

4. Acquisition and genotypic identification of mice

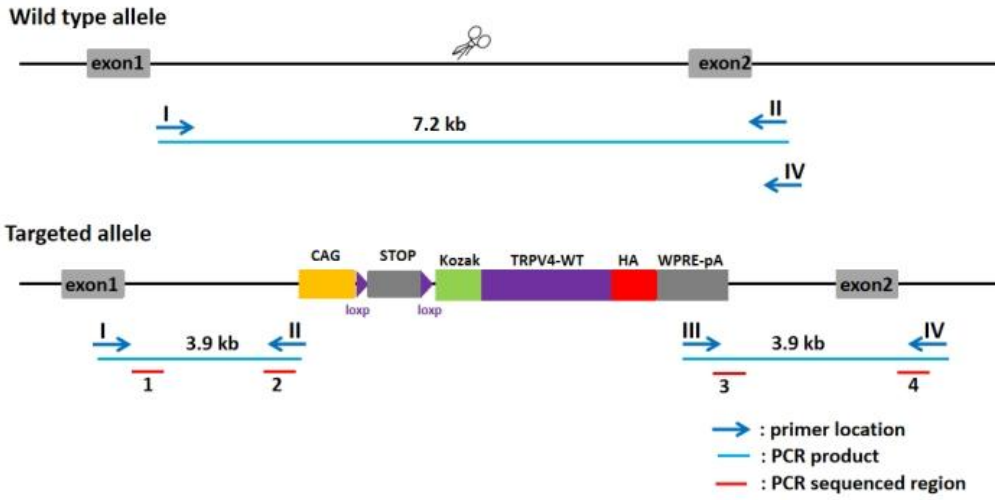
4.1 F0 mice genotyping

The injected fertilized eggs were transplanted into pseudo-pregnant mice, and the mice born about 20 days were F0 generation mice. The genotype were identified by PCR amplification and sequencing.Because the early cleavage rate of fertilized eggs is very fast, the F0 generation mice obtained are chimera and do not necessarily have the ability of stable inheritance. It is necessary to pass the positive F0 generation mice to obtain stable heritable F1 generation mice.

4.2 F1 mice genotyping

F0 generation positive mice were mated with wild type C57BL/6J mice to obtain F1 generation mice. The genotype of F1 generation mice were identified by PCR method and sequencing.

4.2.1 Genotyping strategy of F1 mice



Strategy of F1 mice genotyping

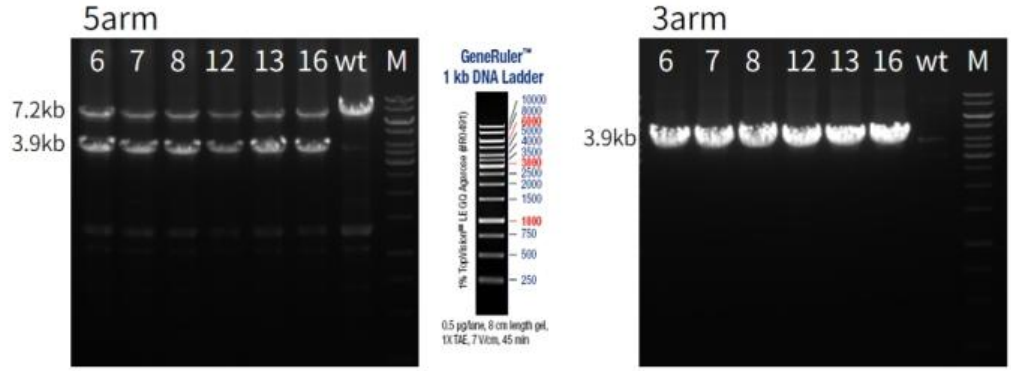
Genotyping method:

5'homologous arm: 3.9 kb fragment should be amplified in the homologous recombinant DNA, and 7.2 kb fragment can be amplified in the negative DNA.

3'homologous arm: 3.9 kb fragment should be amplified in the homologous recombinant DNA, and none of fragments can be amplified in the negative DNA.

4.2.2 PCR genotyping of homologous recombinant F1 mice

The number of homologous recombinant F1 mice were 6,7,8,12,13,16. The agarose gel electrophoresis of PCR results were shown in Figure 3. All positive PCR products were confirmed by sequencing.



Agarose gel electrophoresis of PCR results.
（number：the number of F1 mice；M：1kb DNA ladder）

4.2.3 Method for identifying 5'homologous recombinant F1 mice

Primers：

Primer	Sequence 5' --> 3'	Primer Type
I	TTCTGTGAGACAGCCGGGTA	Forward
II	TTTTTGGGGGTGATGGTGGTC	Reverse

Reaction system:

Reaction Component	Volume (μl)
ddH2O	8.05
2xPCR Buffer	10
Primer I (20pmol/μl)	0.3
Primer II (20pmol/μl)	0.3
KOD-Multi&Epi-*	0.35
Genomic DNA	1
Total	20

* KOD-Multi&Epi-（TOYOBO，Code No：KME-101）

PCR program：

Step #	Temp (°C)	Time	Note
1	94	3 min	-
2	98	20 sec	-
3	63	20 sec	-
4	68	4 min	repeat steps 2-4 for 35cycles
5	68	5 min	-
6	12	-	hold

4.2.4 Method for identifying 3'homologous recombinant F1 mice

Primers：

Primer	Sequence 5' --> 3'	Primer Type
III	TTGCCAGCCATCTGTTGTT	Forward
IV	TGCCACCTTTCACTTAGTTTGT	Reverse

Reaction system:

Reaction Component	Volume (μl)
ddH2O	8.05
2xPCR Buffer	10
Primer III (20pmol/μl)	0.3
Primer IV (20pmol/μl)	0.3
KOD-Multi&Epi-*	0.35
Genomic DNA	1
Total	20

* KOD-Multi&Epi-（TOYOBO，Code No：KME-101）

PCR program：

Step #	Temp (°C)	Time	Note
1	94	3 min	-
2	98	20 sec	-
3	63	20 sec	-
4	68	4 min	repeat steps 2-4 for 35cycles
5	68	5 min	-
6	12	-	hold

4.2.5 Information of homologous recombinant F1 mice

Table：The information of positive F1 mice

Mice ID	DOB	Generations	Sex	Type	Genotype	Father	Mother
6	2023/9/11	F1	♂	KI	He	9F0	6J
7	2023/9/11	F1	♂	KI	He	9F0	6J
8	2023/9/11	F1	♂	KI	He	9F0	6J
12	2023/9/11	F1	♂	KI	He	9F0	6J
13	2023/9/11	F1	♀	KI	He	9F0	6J
16	2023/9/11	F1	♀	KI	He	9F0	6J

SMOC-Report for Rosa26 knock-in mouse model (TRPV4-K608R)

Contents

5. Background and Objective

5.1 Objective

Generation of the CAG-LSL-TRPV4-K608R-HA-WPRE-pA cassette knock-in mouse model at Rosa26 gene locus via CRISPR/Cas9 technology.

5.2 Background

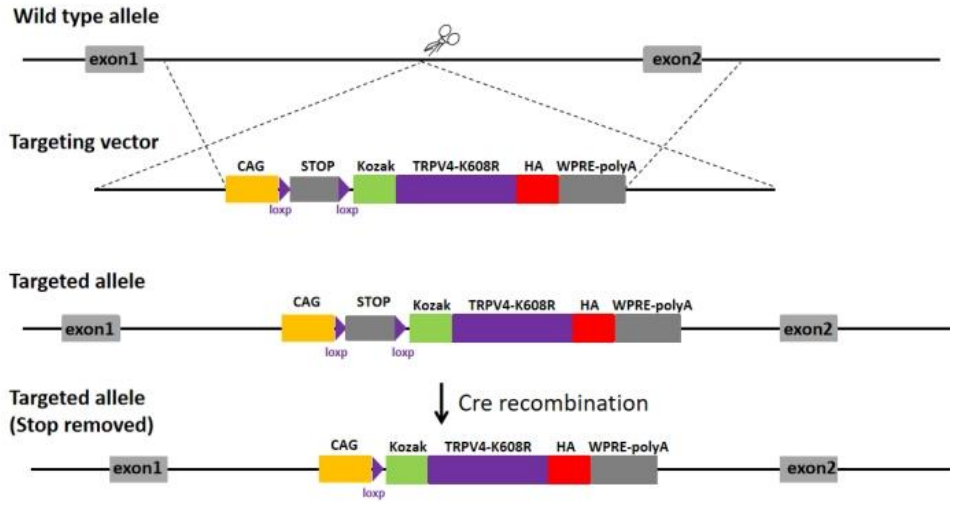
Gene Name (MGI Number): Gt(ROSA)26Sor (104735)
Gene URL Link (MGI) : http://www.informatics.jax.org/marker/MGI:104735
Gene URL Link (Ensembl) : http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000086429;r=6:113067428-113077333
Knockin Cassette: CAG-LSL-TRPV4-K608R-HA-WPRE-pA

6. Abstract

The project process was as follows: 1) Cas9 mRNA and gRNA were produced by in vitro transcription; 2) donor vectors were constructed by in-fusion. The plasmid structure contains 5'homologous arm (3.3 kb), Knock-in, 3'homologous arm (3.3 kb) ; 3) the mixture of Cas9 mRNA,gRNA and donor vector was micro-injected into fertilized eggs (C57BL/6J), then 1 F0 mouse that identified by PCR and sequencing was generated; 4) F0 positive mouse was crossed with wild-type C57BL/6J mice to generate 9 F1 mice.

7. Recombinant strategy

7.1 Strategy figure：



Strategy figure

7.2 Sequence of sgRNAs

gRNAs	Sequence (5'-3')
gRNA1	GGGGACACACTAAGGGAGCT TGG

8. Acquisition and genotypic identification of mice

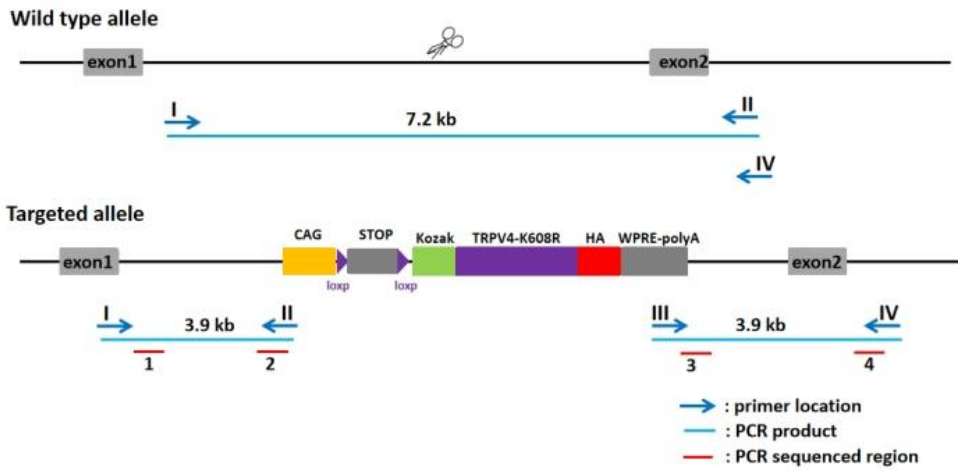
8.1 F0 mice genotyping

The injected fertilized eggs were transplanted into pseudo-pregnant mice, and the mice born about 20 days were F0 generation mice. The genotype were identified by PCR amplification and sequencing.Because the early cleavage rate of fertilized eggs is very fast, the F0 generation mice obtained are chimera and do not necessarily have the ability of stable inheritance. It is necessary to pass the positive F0 generation mice to obtain stable heritable F1 generation mice.

8.2 F1 mice genotyping

F0 generation positive mice were mated with wild type C57BL/6J mice to obtain F1 generation mice. The genotype of F1 generation mice were identified by PCR method and sequencing.

8.2.1 Genotyping strategy of F1 mice



Strategy of F1 mice genotyping

Genotyping method:

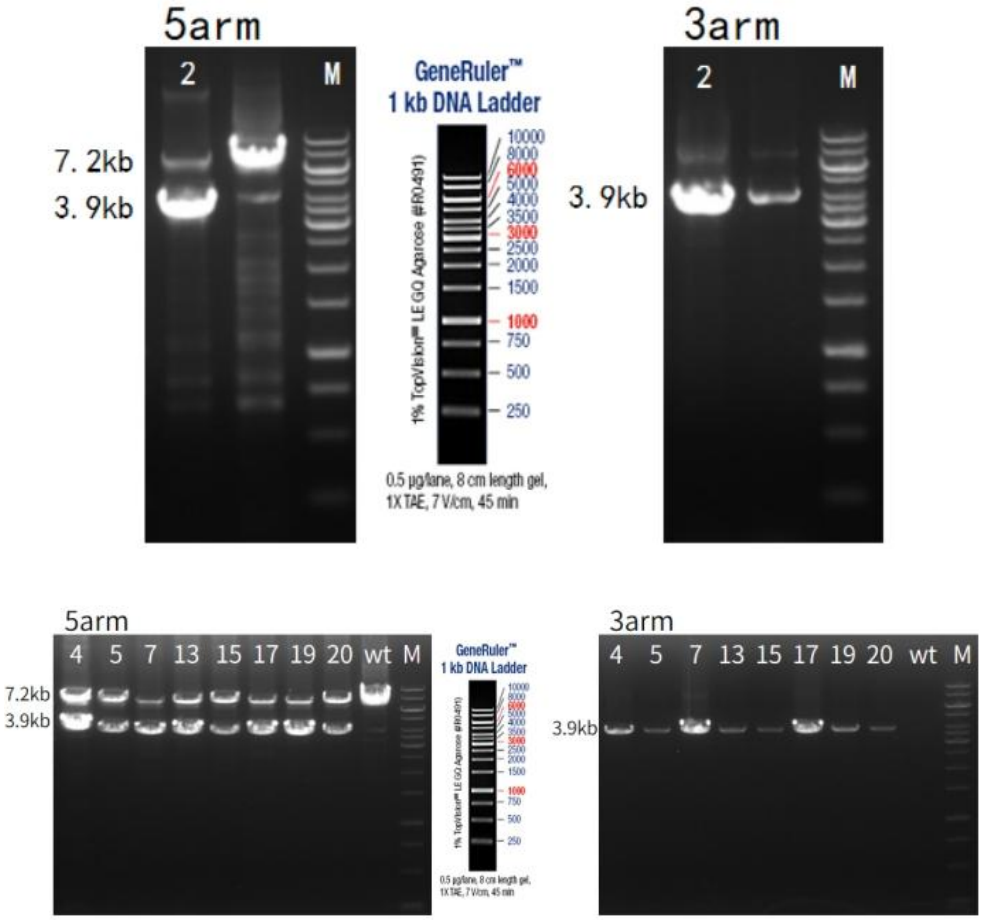
5'homologous arm: 3.9 kb fragment should be amplified in the homologous recombinant DNA, and 7.2 kb fragment can be amplified in the negative DNA.

3'homologous arm: 3.9 kb fragment should be amplified in the homologous recombinant DNA, none of fragments can be amplified in the negative DNA.

8.2.2 PCR genotyping of homologous recombinant F1 mice

The number of homologous recombinant F1 mice were 2,4,5,7,13,15,17,19,20. The agarose

gel electrophoresis of PCR results were shown in Figure 3. All positive PCR products were confirmed by sequencing.



Agarose gel electrophoresis of PCR results.

（number：the number of F1 mice；M：1kb DNA ladder）

8.2.3 Method for identifying 5'homologous recombinant F1 mice

Primers：

Primer	Sequence 5' --> 3'	Primer Type
I	TTCTGTGAGACAGCCGGGTA	Forward
II	TTTTTGGGGGTGATGGTGGTC	Reverse

Reaction system:

Reaction Component	Volume (μl)
ddH2O	8.05
2xPCR Buffer	10
Primer I (20pmol/μl)	0.3
Primer II (20pmol/μl)	0.3
KOD-Multi&Epi-*	0.35
Genomic DNA	1
Total	20

* KOD-Multi&Epi-（TOYOBO，Code No：KME-101）

PCR program：

Step #	Temp (°C)	Time	Note
1	94	3 min	-
2	98	20 sec	-
3	63	20 sec	-
4	68	4 min	repeat steps 2-4 for 35cycles
5	68	5 min	-
6	12	-	hold

8.2.4 Method for identifying 3'homologous recombinant F1 mice

Primers：

Primer	Sequence 5' --> 3'	Primer Type
III	TTGCCAGCCATCTGTTGTT	Forward
IV	TGCCACCTTTCACTTAGTTTGT	Reverse

Reaction system:

Reaction Component	Volume (μl)
ddH2O	8.05
2xPCR Buffer	10
Primer III (20pmol/μl)	0.3
Primer IV (20pmol/μl)	0.3
KOD-Multi&Epi-*	0.35
Genomic DNA	1
Total	20

* KOD-Multi&Epi-（TOYOBO，Code No：KME-101）

PCR program：

Step #	Temp (°C)	Time	Note
1	94	3 min	-
2	98	20 sec	-
3	63	20 sec	-
4	68	4 min	repeat steps 2-4 for 35cycles
5	68	5 min	-
6	12	-	hold

8.2.5 Information of homologous recombinant F1 mice

Table：The information of positive F1 mice

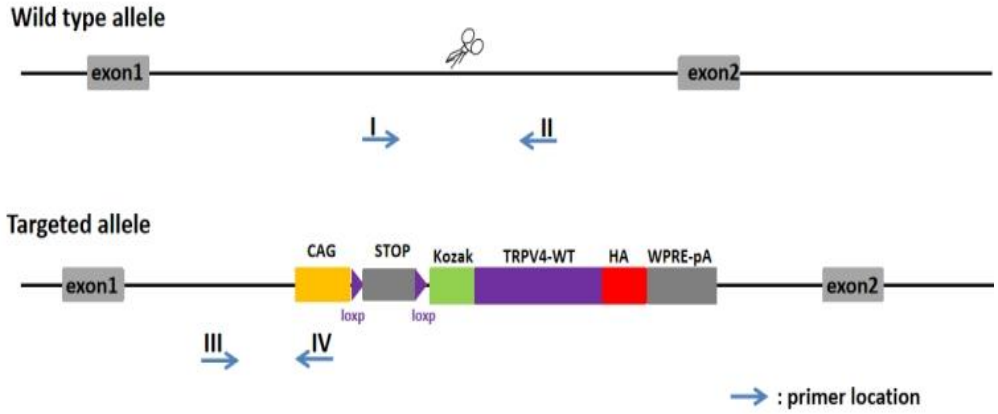
Mice ID	DOB	Generations	Sex	Type	Genotype	Father	Mother

2	2023/8/7	F1	♀	KI	He	3F0	6J
4	2023/8/14	F1	♂	KI	He	3F0	6J
5	2023/8/14	F1	♂	KI	He	3F0	6J
7	2023/8/14	F1	♂	KI	He	3F0	6J
13	2023/8/14	F1	♂	KI	He	3F0	6J
15	2023/8/14	F1	♀	KI	He	3F0	6J
17	2023/8/14	F1	♀	KI	He	3F0	6J
19	2023/8/14	F1	♀	KI	He	3F0	6J
20	2023/8/14	F1	♀	KI	He	3F0	6J

Rosa26-LSL-TRPV4-WT-HA Genotyping Protocol

Common Name	Rosa26-LSL-TRPV4-WT-HA	Cat. NO.	
Strain of Origin	C57BL/6J	Version	V1

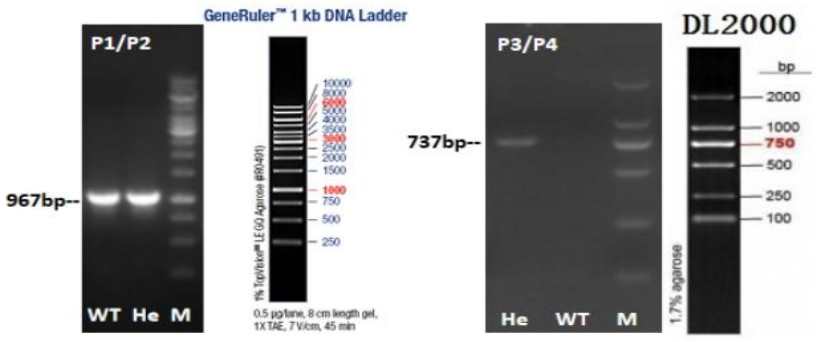
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCAGATTCTTTTATAGGGGACACA	Forward
P2	TAAAGGCCACTCAATGCTCACTAA	Reverse
P3	GGGTGGGGTTGGGAAATCTT	Forward
P4	TAGGGGGCGTACTTGGCATA	Reverse

Expected results

Results	
Genotype	Wild type：P1P2 =967 bp； Heterozygote：P1P2 =967 bp；P3P4=737 bp Homozygote：P3P4 =737 bp

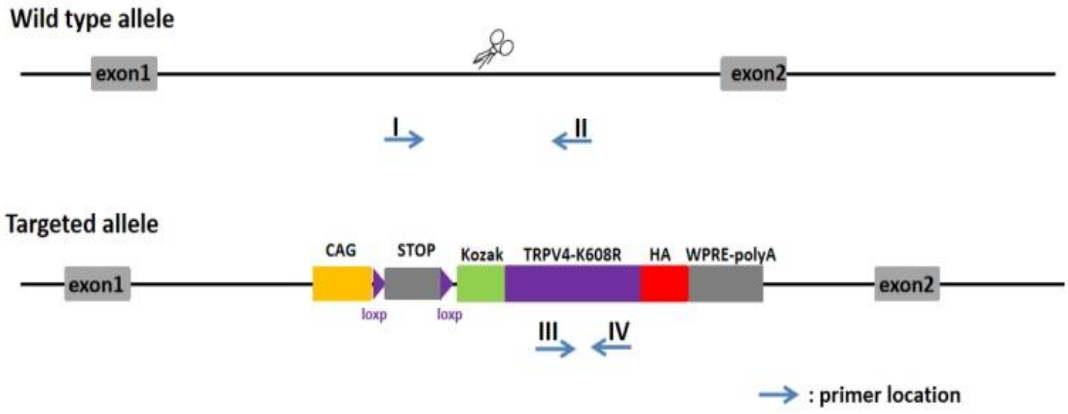
Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH2O			8.0
	2×Taq Plus Master Mix			10.0
	P1(10 pmol/μl) or P3(10 pmol/μl)			0.5
	P2(10 pmol/μl) or P4(10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Taq Plus Master Mix from Vazyme(Code Number: P222-1)			
Cycling Reaction	Step	Temp	Time	Note
	1	95°C	5 min	
	2	95°C	30 sec	
	3	60°C	30 sec	
	4	72°C	30 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	

Rosa26-LSL-TRPV4-K608R-HA Genotyping Protocol

Common Name	Rosa26-LSL-TRPV4-K608R-HA	Cat. NO.	CM-KI-231843
Strain of Origin	C57BL/6J	Version	V1

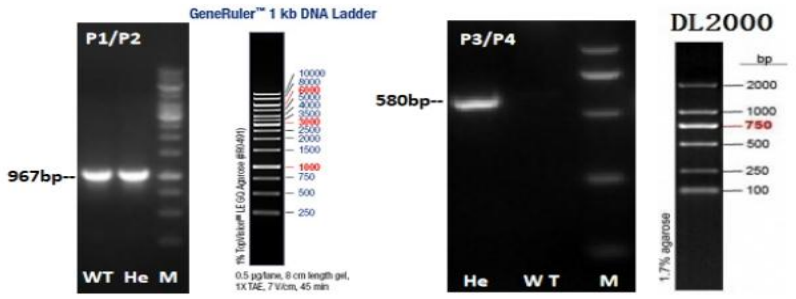
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCAGATTCTTTTATAGGGGACACA	Forward
P2	TAAAGGCCACTCAATGCTCACTAA	Reverse
P3	CCTGTGTGCCATGGTCATCT	Forward
P4	CAGGAGGAAGGTGCTGAAGG	Reverse

Expected results

Results	
Genotype	Wild type: P1P2 =967 bp; Heterozygote: P1P2 =967 bp; P3P4=580 bp Homozygote: P3P4 =580 bp

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH2O			8.0
	2×Taq Plus Master Mix			10.0
	P1(10 pmol/μl) or P3(10 pmol/μl)			0.5
	P2(10 pmol/μl) or P4(10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Taq Plus Master Mix from Vazyme(Code Number: P222-1)			
Cycling Reaction	Step	Temp	Time	Note
	1	95°C	5 min	
	2	95°C	30 sec	
	3	60°C	30 sec	
	4	72°C	30 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	

Uncropped gels for Fig. 1 to 5 and Fig. S1, 4, 9 to 12, 15 to 18. After electrophoretic transfer of proteins, the PVDF membranes were cut into strips containing sets of samples, and were then subjected to immunoblotting. Show here are films that had been exposed and developed to the membrane strips. The Pierce Prestained Protein MW Marker (Cat. 26612, ThermoFisher Scientific) was used as the protein markers.

Fig. 1A

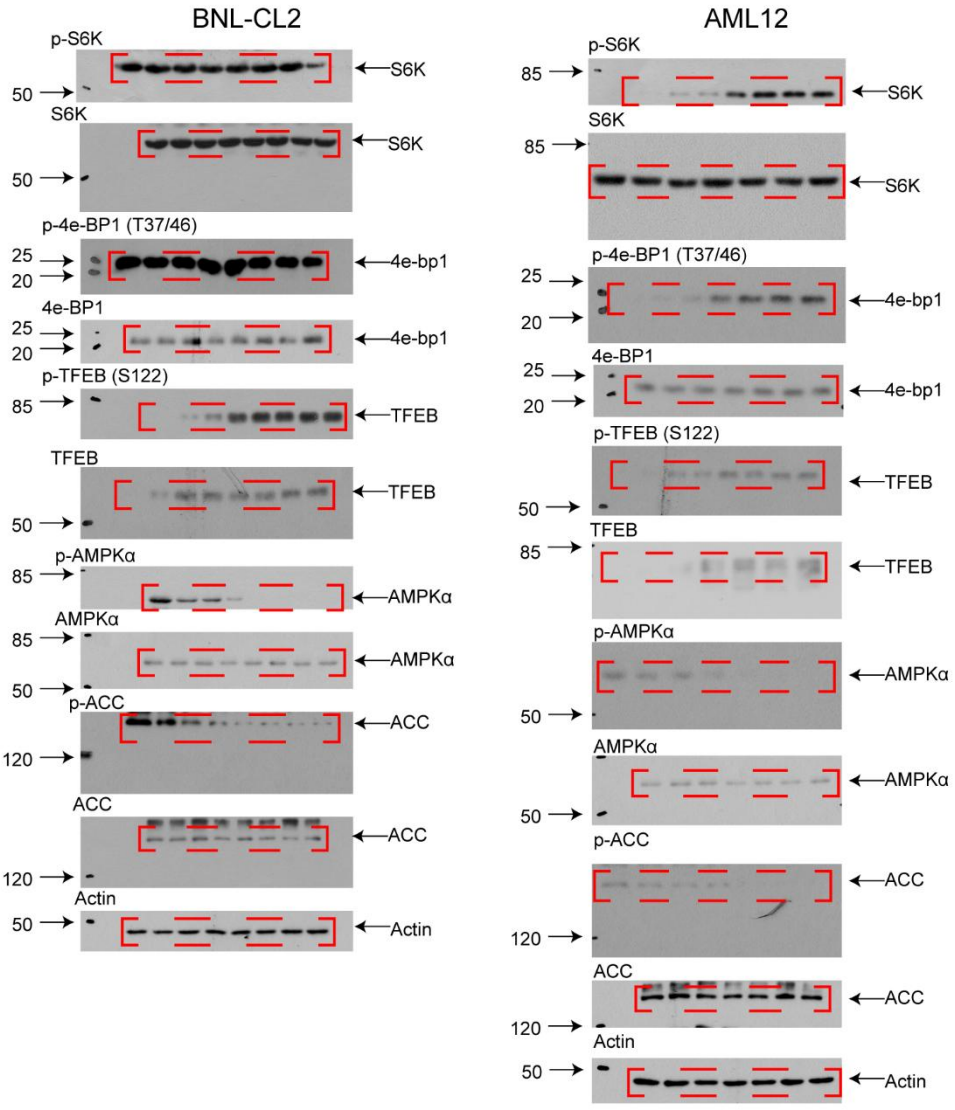

BNL-CL2
p-S6K
S6K
50
S6K
S6K
50
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-TFEB (S122)
85
TFEB
TFEB
50
TFEB
p-AMPKα
85
AMPKα
AMPKα
85
AMPKα
50
p-ACC
120
ACC
ACC
120
ACC
Actin
50
Actin
AML12
p-S6K
85
S6K
S6K
85
S6K
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-TFEB (S122)
50
TFEB
TFEB
85
TFEB
p-AMPKα
50
AMPKα
AMPKα
50
AMPKα
p-ACC
120
ACC
ACC
120
ACC
Actin
50
Actin

Fig. 1A

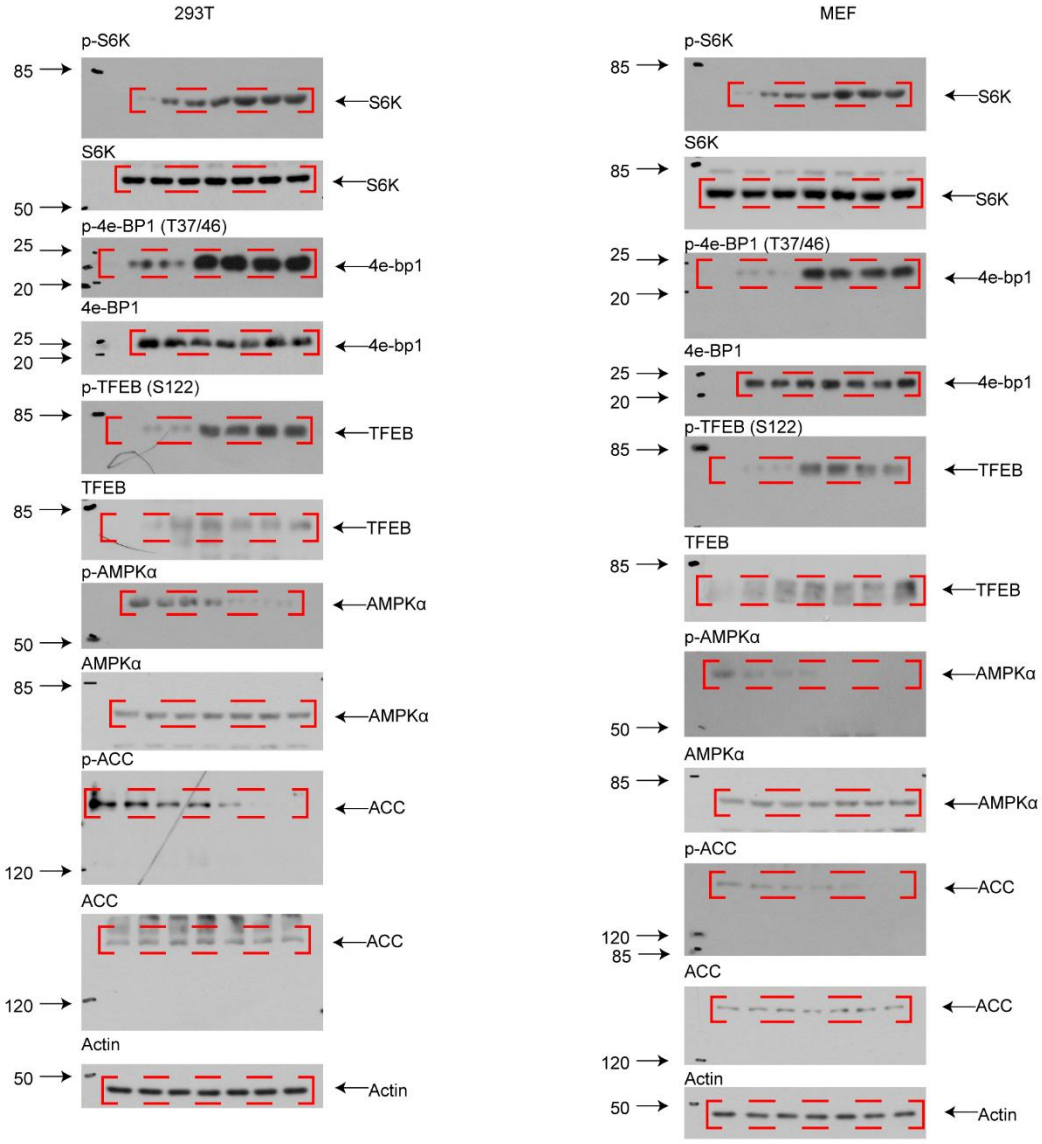

293T
MEF
p-S6K
S6K
p-4e-BP1 (T37/46)
4e-BP1
p-TFEB (S122)
TFEB
p-AMPKα
AMPKα
p-ACC
ACC
Actin
85
50
25
20
120
S6K
4e-bp1
TFEB
AMPKα
ACC
Actin

Fig. 1B

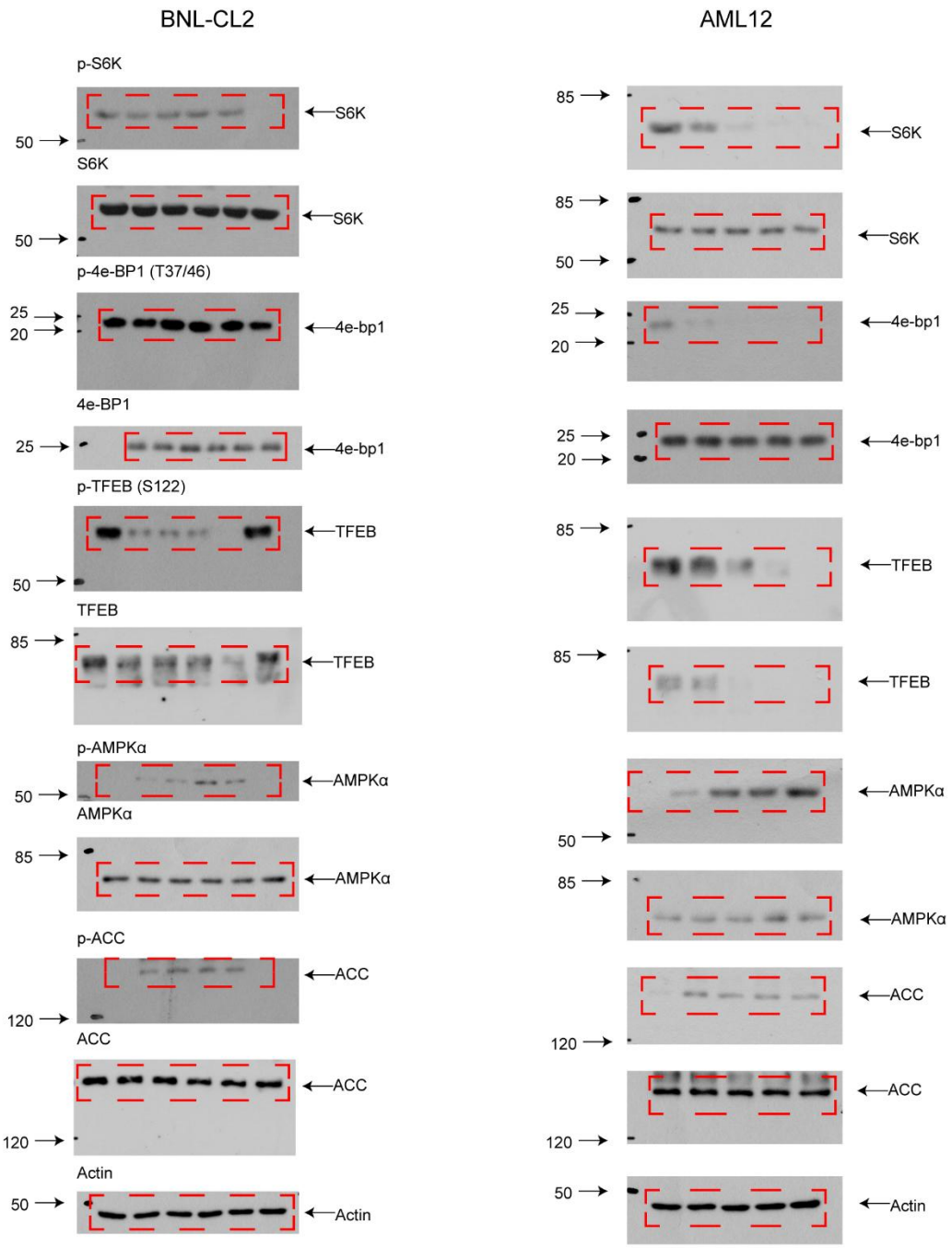

BNL-CL2
AML12
p-S6K
S6K
p-4e-BP1 (T37/46)
4e-BP1
p-TFEB (S122)
TFEB
p-AMPKα
AMPKα
p-ACC
ACC
Actin
50
85
25
20
120
←S6K
←4e-bp1
←TFEB
←AMPKα
←ACC
←Actin

Fig. 1B

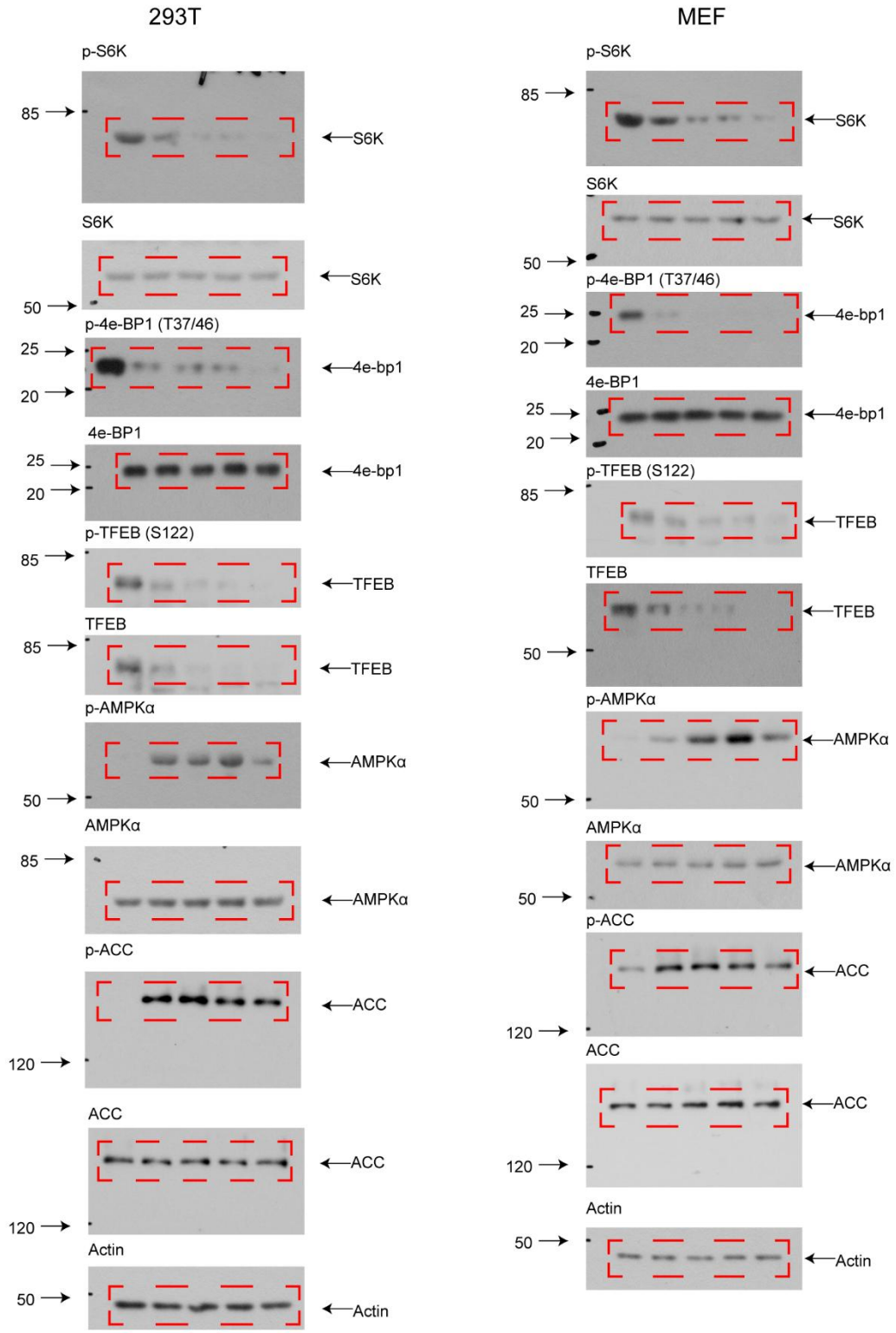

293T
MEF
p-S6K
S6K
p-4e-BP1 (T37/46)
4e-BP1
p-TFEB (S122)
TFEB
p-AMPKα
AMPKα
p-ACC
ACC
Actin
85
50
25
20
120
←S6K
←4e-bp1
←TFEB
←AMPKα
←ACC
←Actin

Fig. 1C

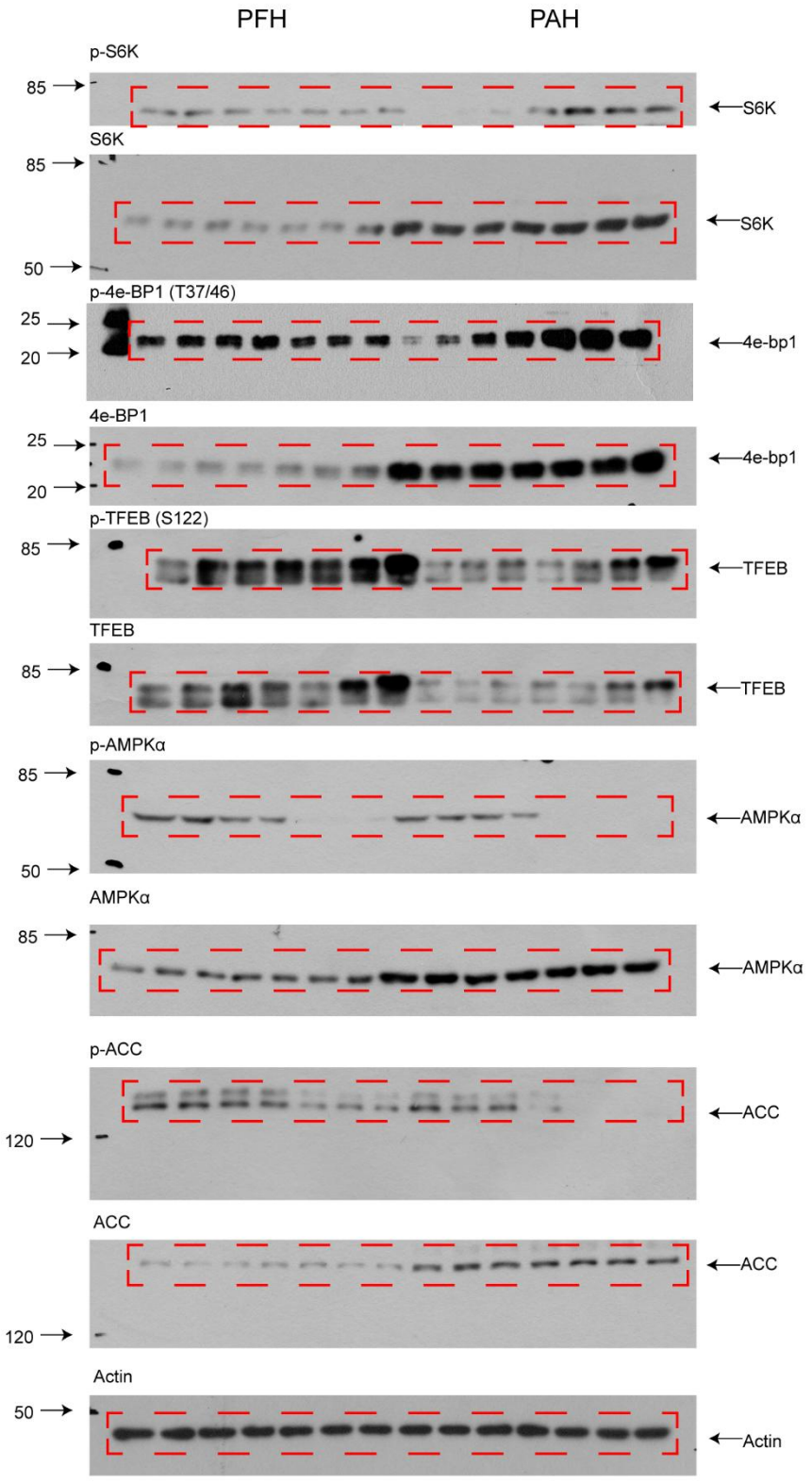

PFH
PAH
p-S6K
85
S6K
S6K
85
50
S6K
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-TFEB (S122)
85
TFEB
TFEB
85
TFEB
p-AMPKα
85
50
AMPKα
AMPKα
85
AMPKα
p-ACC
120
ACC
ACC
120
ACC
Actin
50
Actin

Fig. 1D

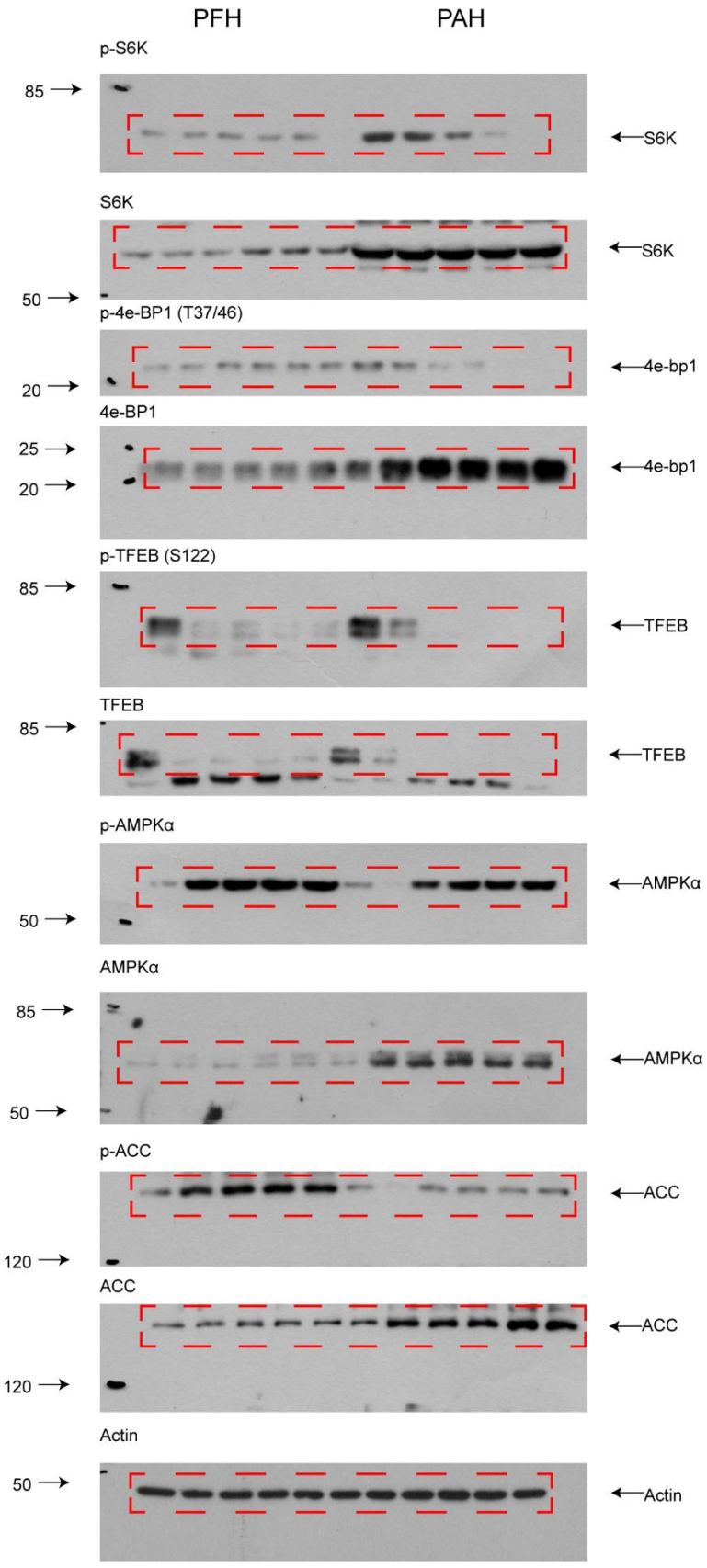

PFH
PAH
p-S6K
85
S6K
S6K
50
p-4e-BP1 (T37/46)
4e-bp1
20
4e-BP1
25
20
4e-bp1
p-TFEB (S122)
85
TFEB
TFEB
85
TFEB
p-AMPKα
AMPKα
50
AMPKα
85
50
AMPKα
p-ACC
ACC
120
ACC
ACC
120
Actin
Actin
50

Fig. 1E

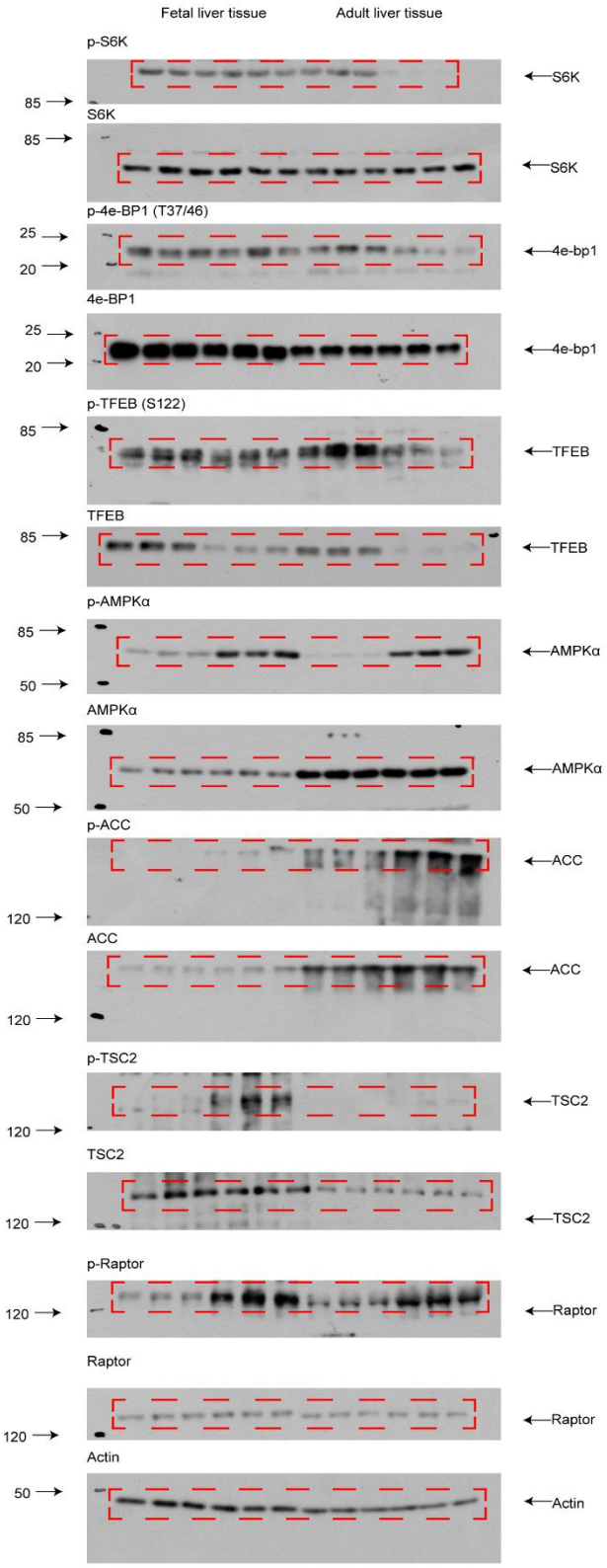

Fetal liver tissue
Adult liver tissue
p-S6K
S6K
85
S6K
85
S6K
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-TFEB (S122)
85
TFEB
TFEB
85
TFEB
p-AMPKα
85
50
AMPKα
AMPKα
85
50
AMPKα
p-ACC
120
ACC
ACC
120
ACC
p-TSC2
120
TSC2
TSC2
120
TSC2
p-Raptor
120
Raptor
Raptor
120
Raptor
Actin
50
Actin

Fig. 1F

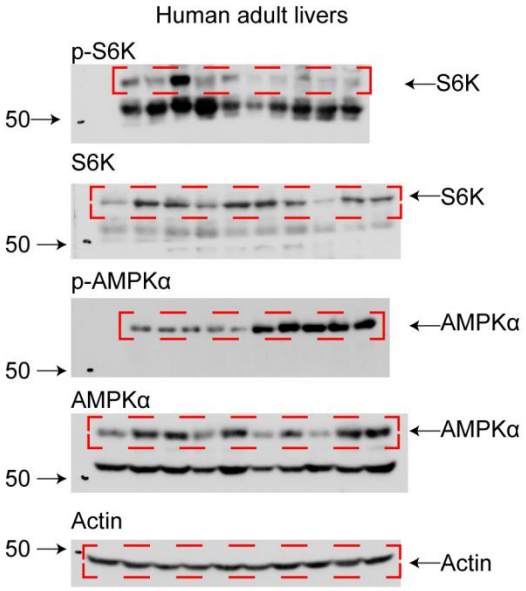

Human adult livers
p-S6K
←S6K
50→
S6K
←S6K
50→
p-AMPKα
←AMPKα
50→
AMPKα
←AMPKα
50→
Actin
50→
←Actin

Fig. 1I

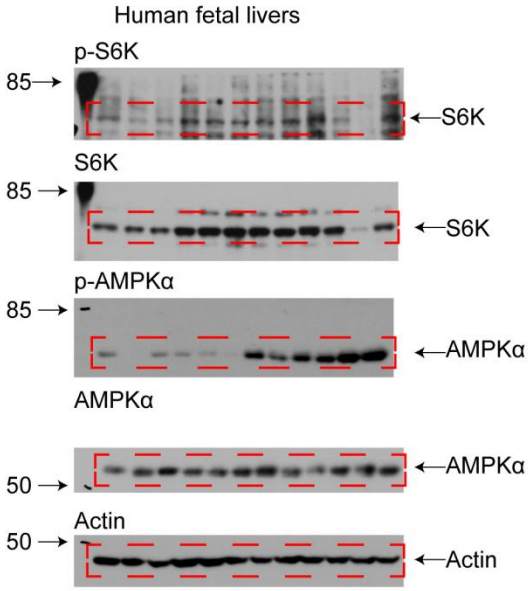

Human fetal livers
p-S6K
85→
←S6K
S6K
85→
←S6K
p-AMPKα
85→
←AMPKα
AMPKα
←AMPKα
50→
Actin
50→
←Actin

Fig. 1L

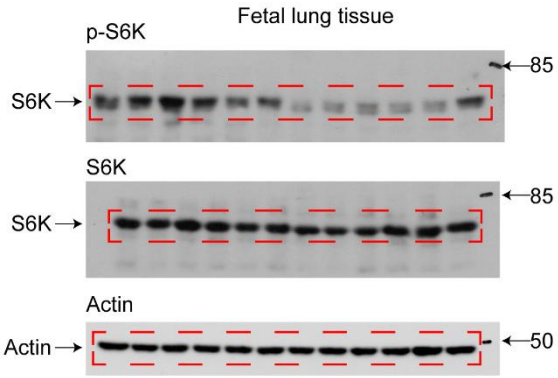


Fig. 1M

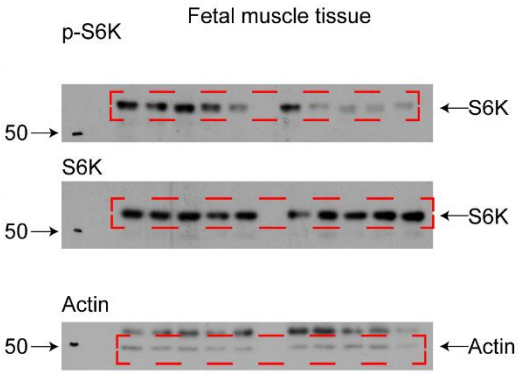


Fig. 1N

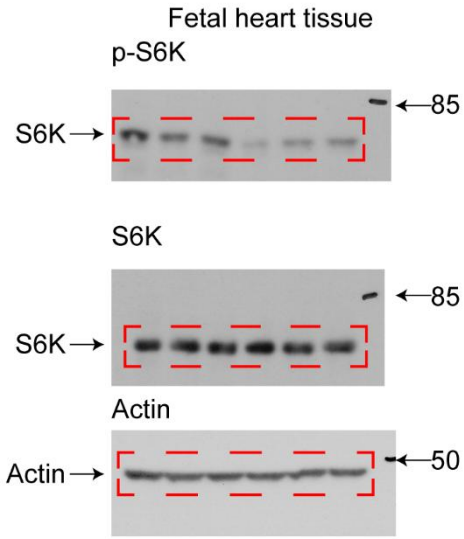


Fig. 2B

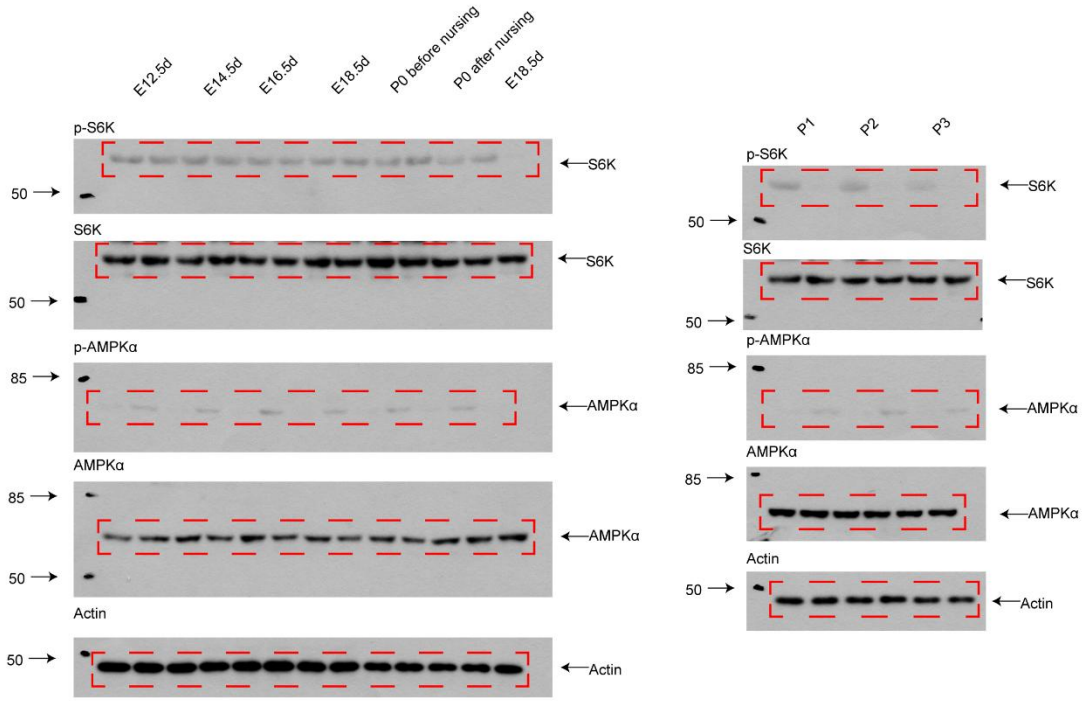

E12.5d
E14.5d
E16.5d
E18.5d
P0 before nursing
P0 after nursing
E18.5d
p-S6K
S6K
50
p-AMPKα
AMPKα
85
Actin
P1
P2
P3

Fig. 2F

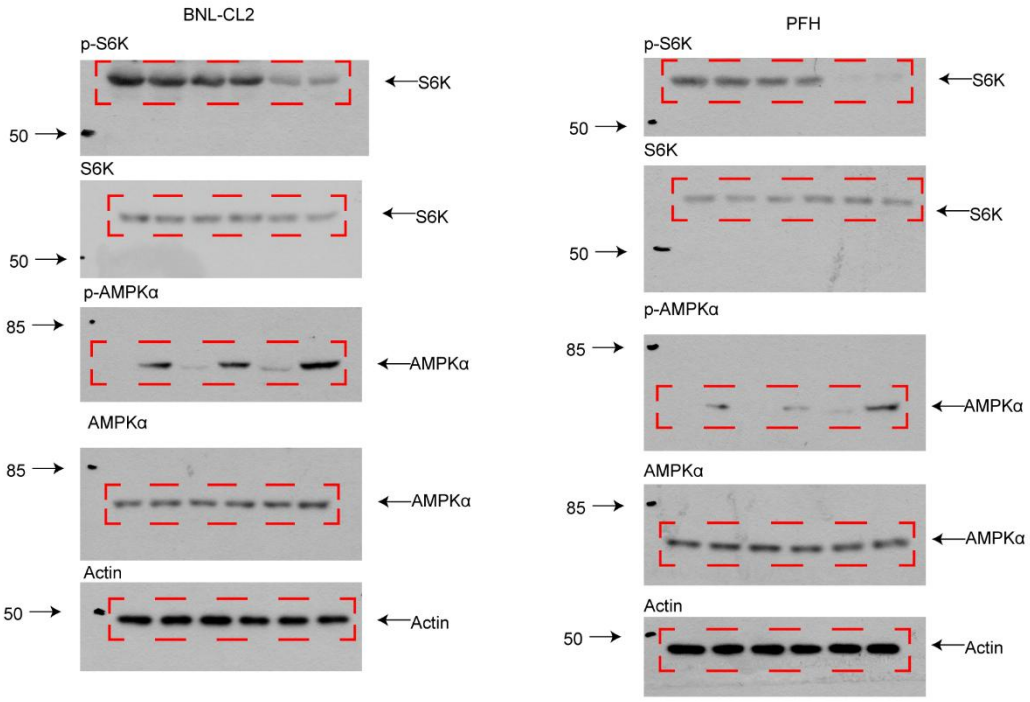

BNL-CL2
p-S6K
S6K
50
S6K
S6K
50
p-AMPKα
85
AMPKα
AMPKα
85
AMPKα
Actin
50
Actin
PFH
p-S6K
S6K
50
S6K
S6K
50
p-AMPKα
85
AMPKα
AMPKα
85
AMPKα
Actin
50
Actin

Fig. 2G

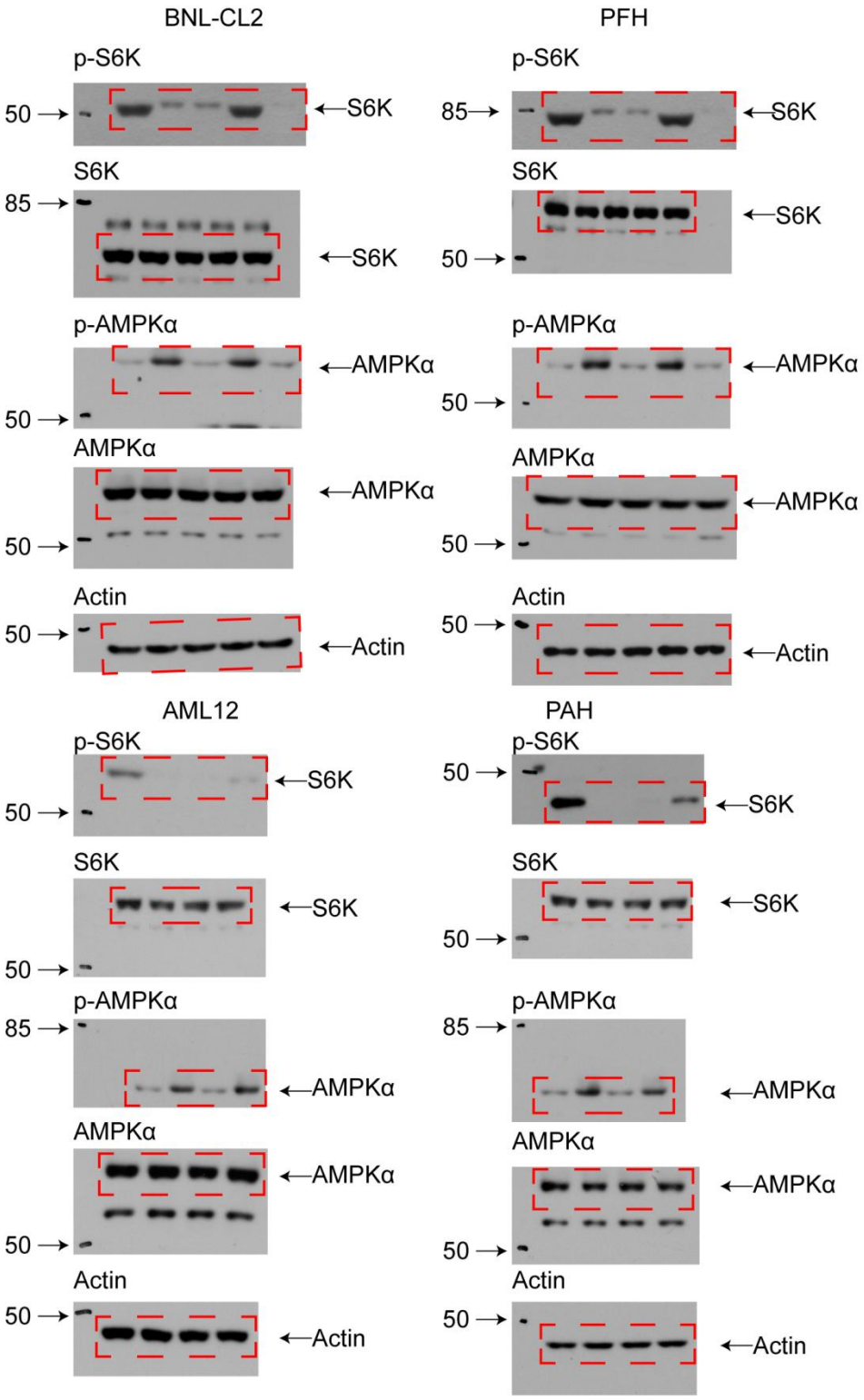

BNL-CL2
p-S6K
50
S6K
S6K
85
S6K
p-AMPKα
AMPKα
50
AMPKα
AMPKα
50
Actin
50
Actin
PFH
p-S6K
85
S6K
S6K
50
S6K
p-AMPKα
AMPKα
50
AMPKα
AMPKα
50
AMPKα
Actin
50
Actin
AML12
p-S6K
50
S6K
S6K
50
S6K
p-AMPKα
85
AMPKα
AMPKα
50
AMPKα
Actin
50
Actin
PAH
p-S6K
50
S6K
S6K
50
S6K
p-AMPKα
85
AMPKα
AMPKα
50
AMPKα
Actin
50
Actin

Fig. 2H

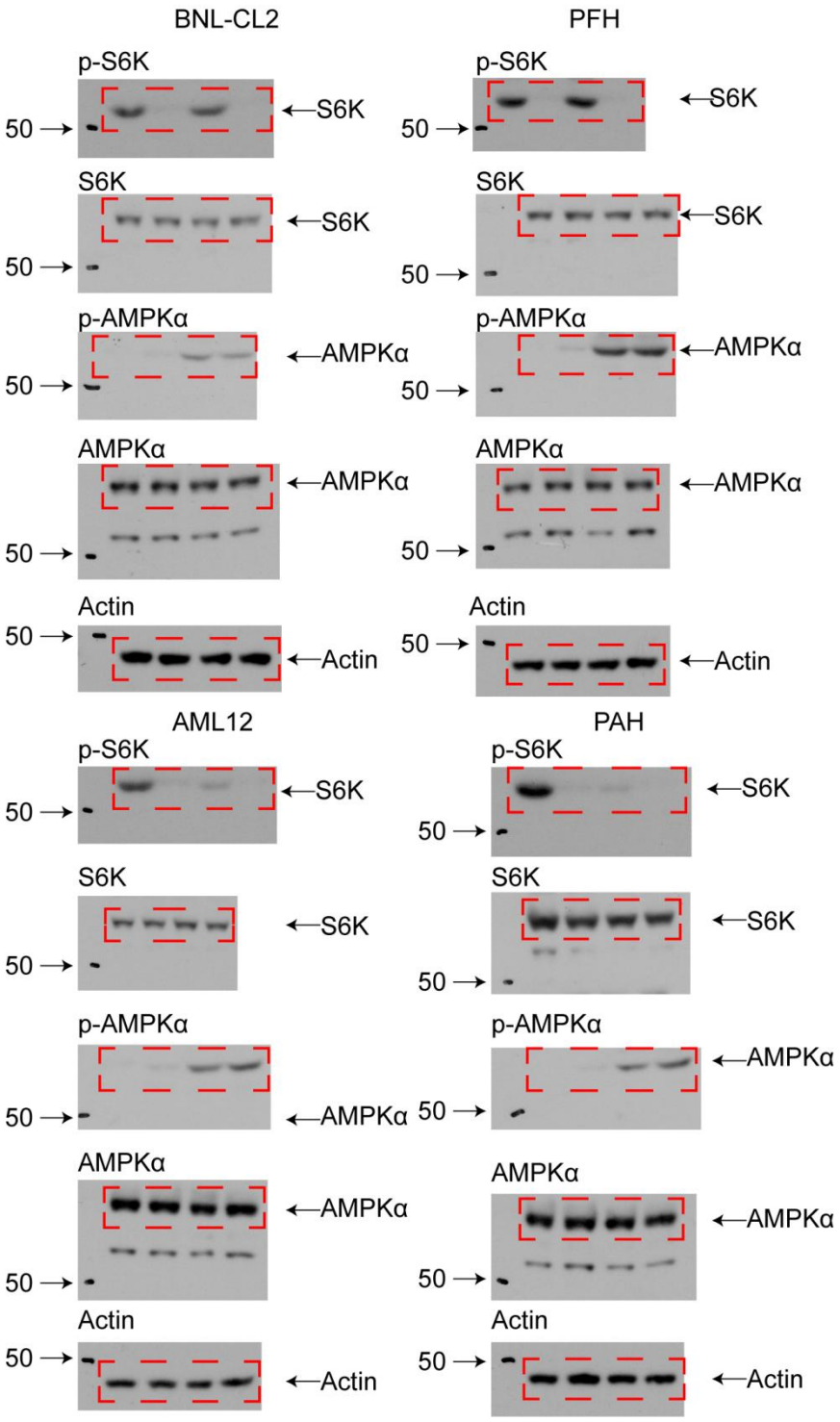


Fig. 2I

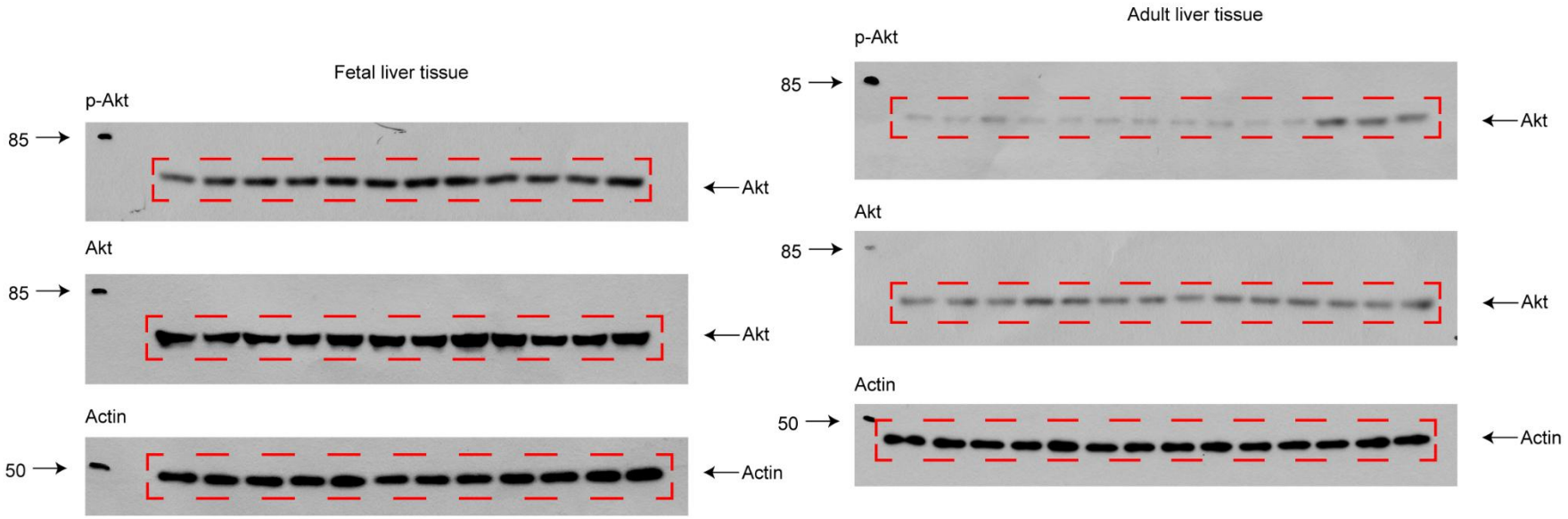

Fetal liver tissue
p-Akt
85
Akt
Akt
85
Akt
Actin
50
Actin
Adult liver tissue
p-Akt
85
Akt
Akt
85
Akt
Actin
50
Actin

Fig. 3A

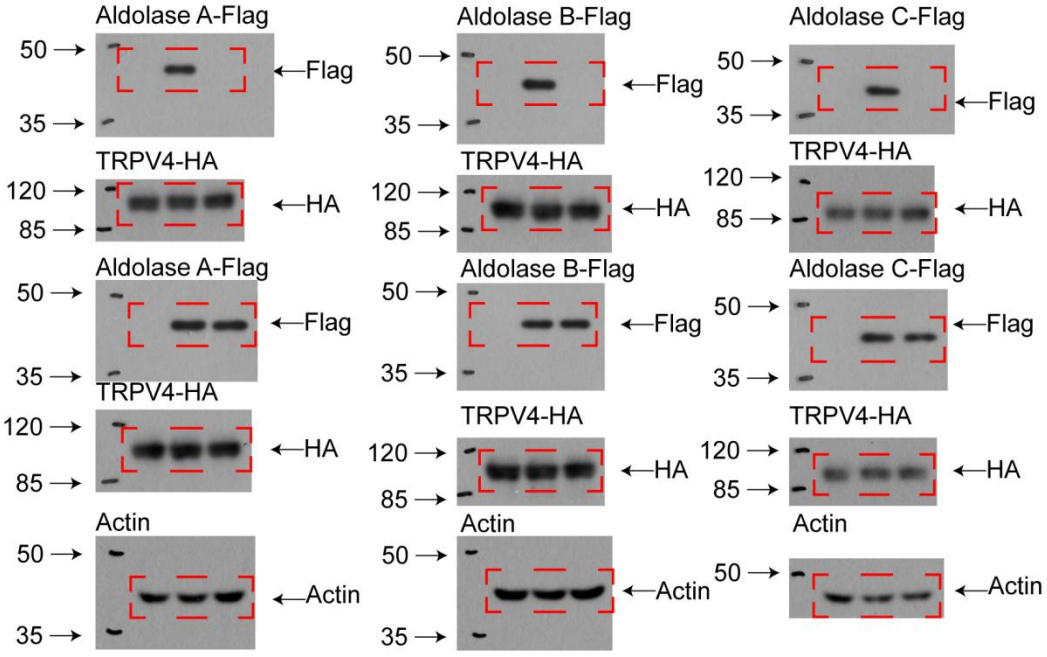

Aldolase A-Flag
50
35
Flag
TRPV4-HA
120
85
HA
Aldolase A-Flag
50
35
Flag
TRPV4-HA
120
85
HA
Actin
50
35
Actin
Aldolase B-Flag
50
35
Flag
TRPV4-HA
120
85
HA
Aldolase B-Flag
50
35
Flag
TRPV4-HA
120
85
HA
Actin
50
35
Actin
Aldolase C-Flag
50
35
Flag
TRPV4-HA
120
85
HA
Aldolase C-Flag
50
35
Flag
TRPV4-HA
120
85
HA
Actin
50
Actin

Fig. 3G

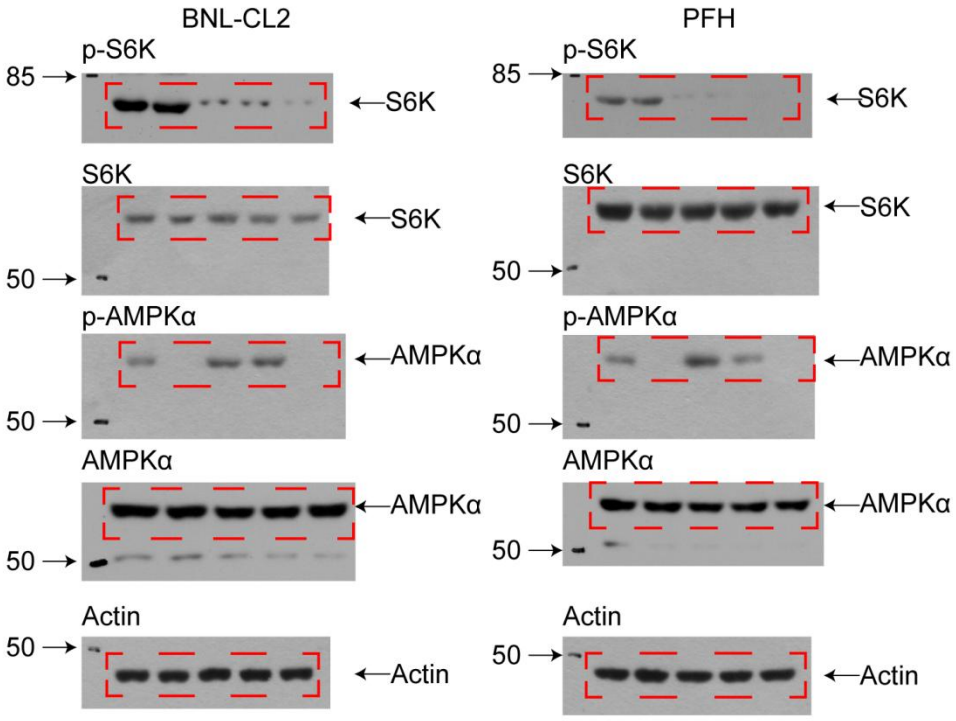


Fig. 3J

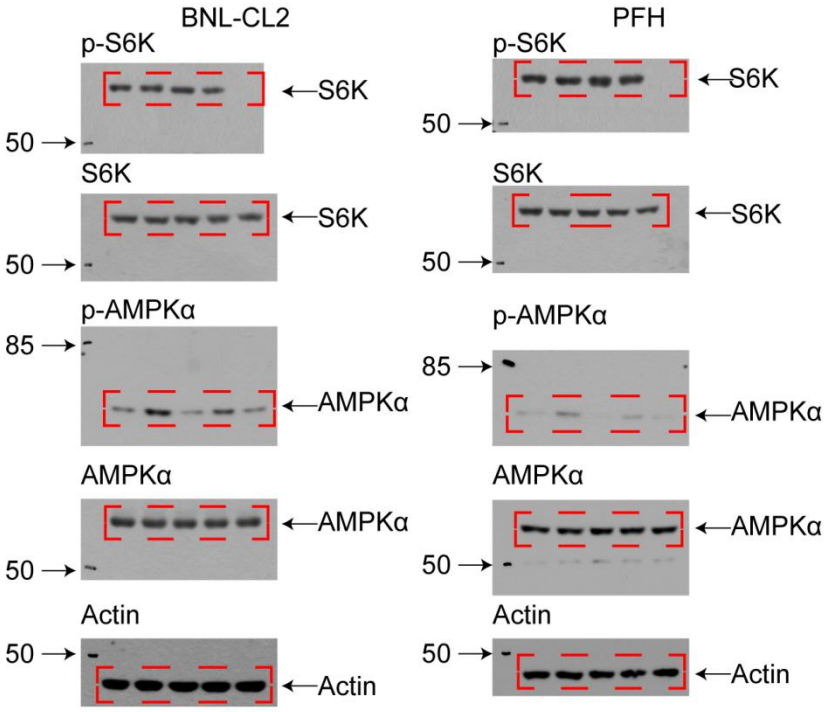

BNL-CL2
PFH
p-S6K
S6K
50
S6K
S6K
50
p-AMPKα
85
AMPKα
AMPKα
AMPKα
50
Actin
50
Actin

Fig. 3K

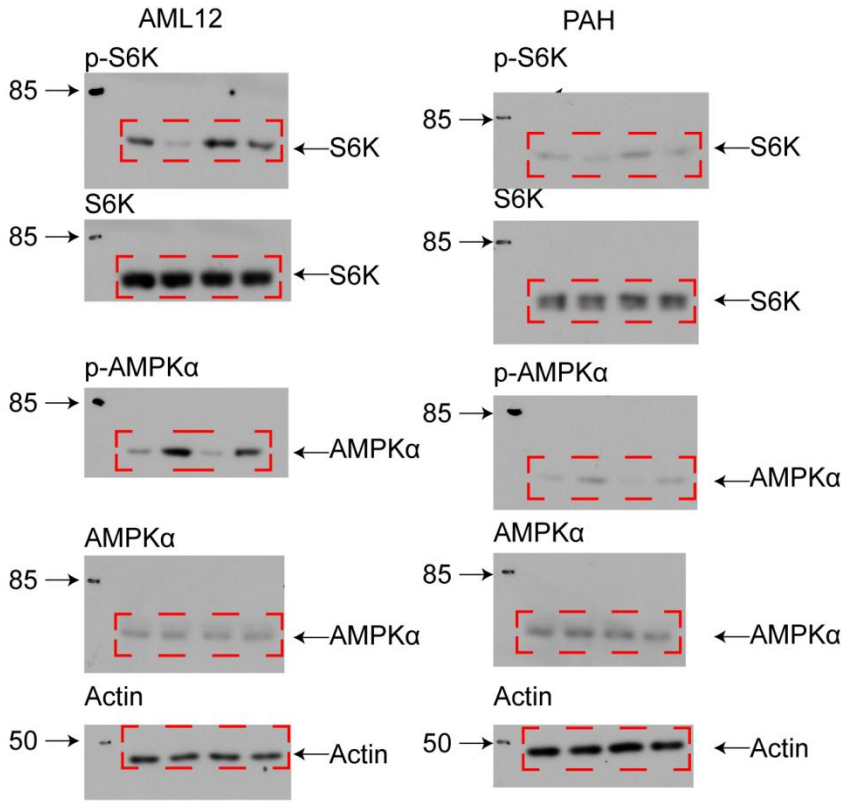


Fig.4A

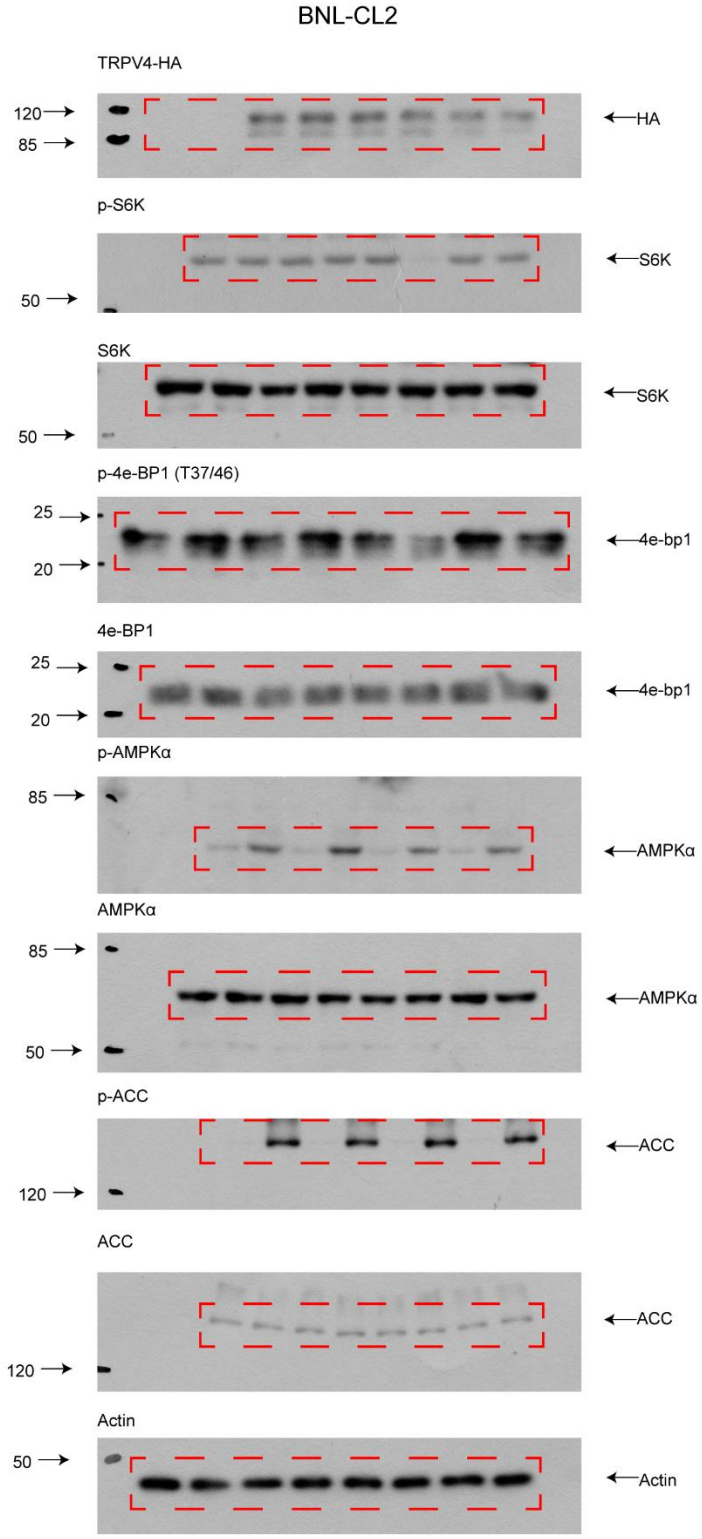

BNL-CL2
TRPV4-HA
120
85
HA
p-S6K
50
S6K
S6K
50
S6K
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-AMPKα
85
AMPKα
AMPKα
85
50
AMPKα
p-ACC
120
ACC
ACC
120
ACC
Actin
50
Actin

Fig. 4F

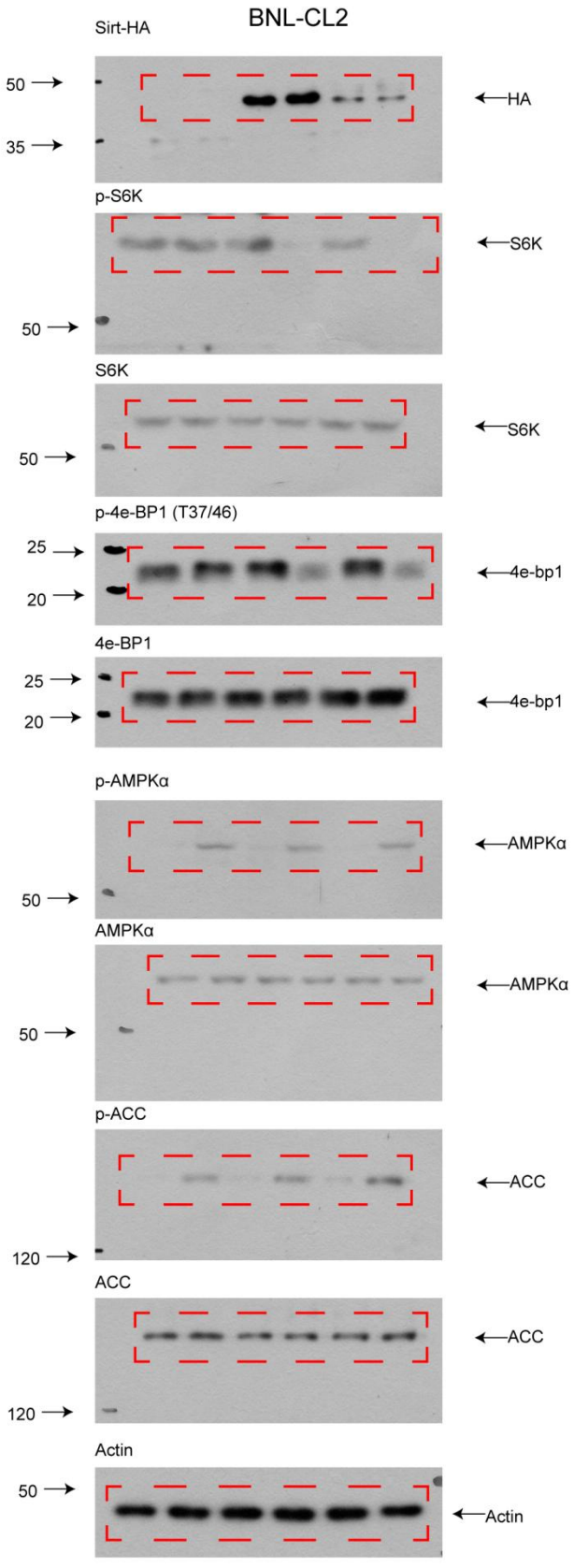

BNL-CL2
Sirt-HA
50
35
HA
p-S6K
S6K
50
S6K
S6K
50
S6K
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-AMPKα
50
AMPKα
AMPKα
50
AMPKα
p-ACC
120
ACC
ACC
120
ACC
Actin
50
Actin

Fig. 4G

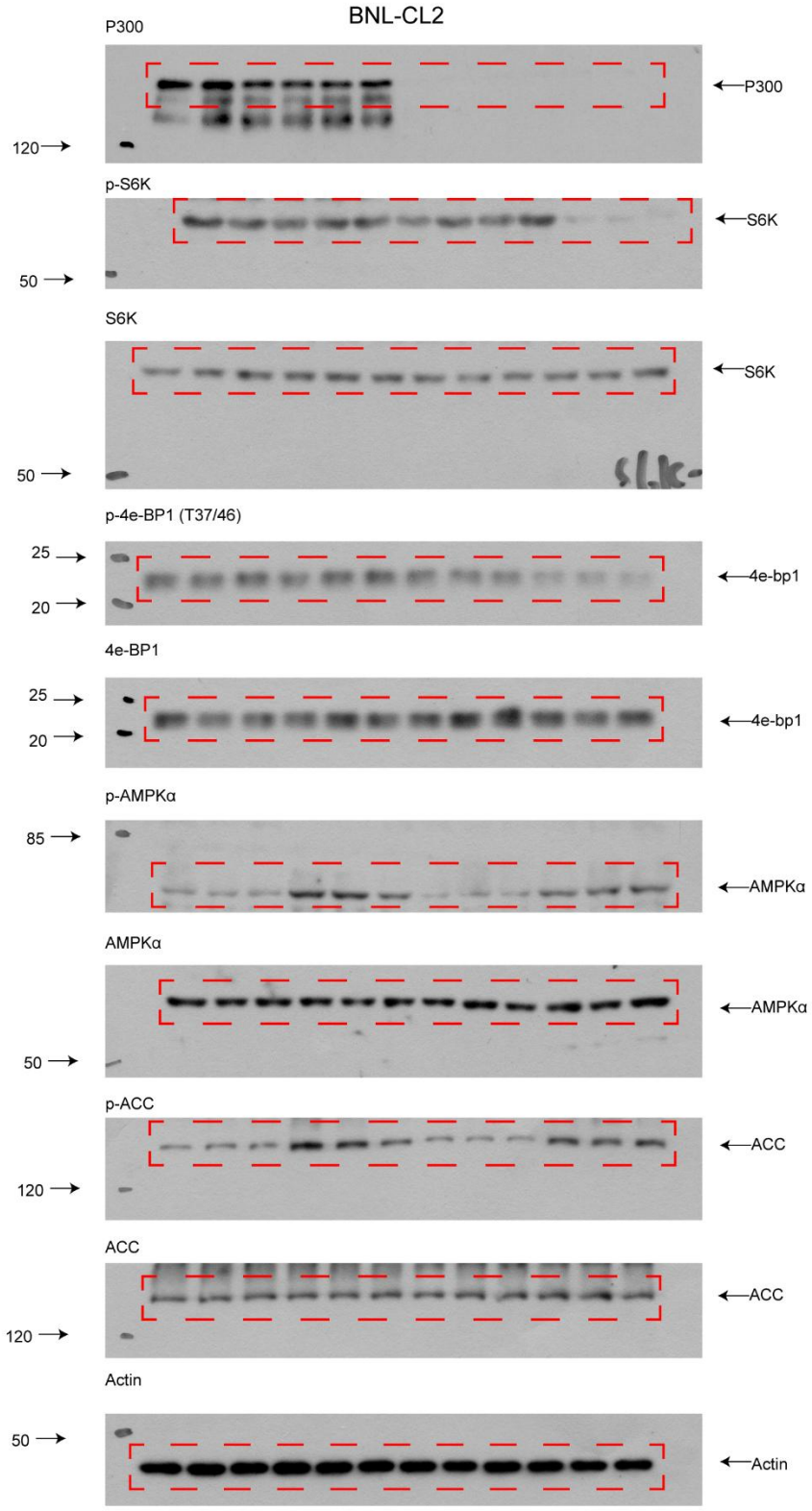

BNL-CL2
P300
P300
120
p-S6K
S6K
50
S6K
S6K
50
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-AMPKα
85
AMPKα
AMPKα
AMPKα
50
p-ACC
ACC
120
ACC
ACC
120
Actin
50
Actin

Fig. 4I

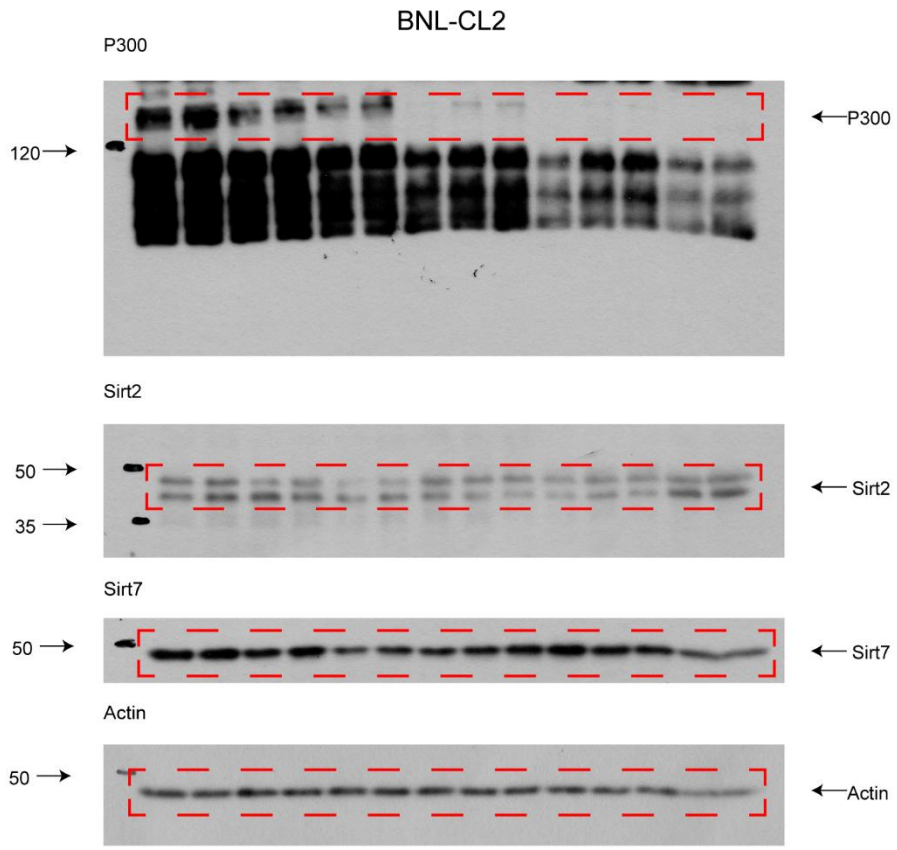

BNL-CL2
P300
120
P300
Sirt2
50
35
Sirt2
Sirt7
50
Sirt7
Actin
50
Actin

Fig. 5A

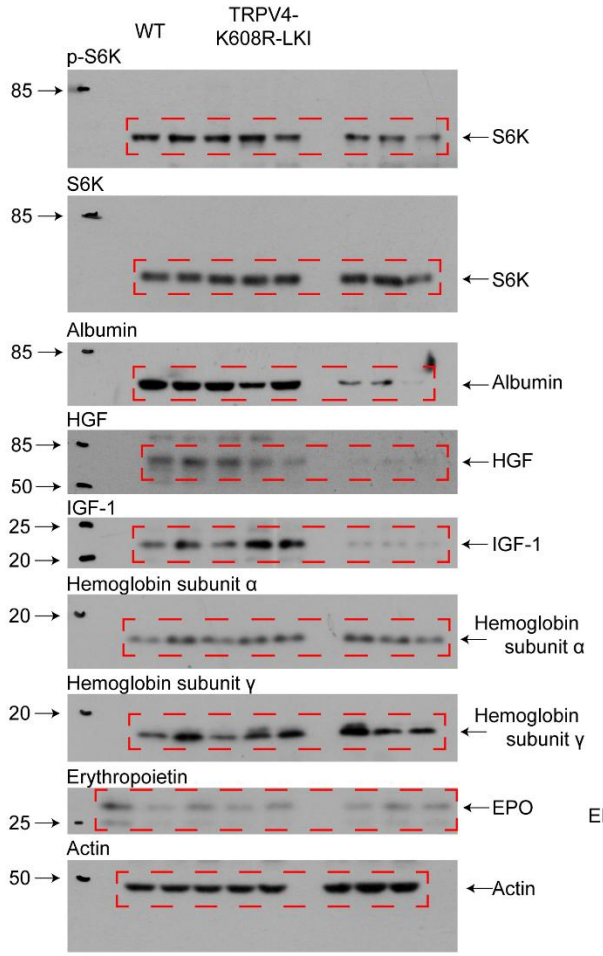

WT
TRPV4-
K608R-LKI
p-S6K
85
S6K
S6K
85
S6K
Albumin
85
Albumin
HGF
85
50
HGF
IGF-1
25
20
IGF-1
Hemoglobin subunit α
20
Hemoglobin
subunit α
Hemoglobin subunit γ
20
Hemoglobin
subunit γ
Erythropoietin
25
EPO
Actin
50
Actin

Fig. 5B

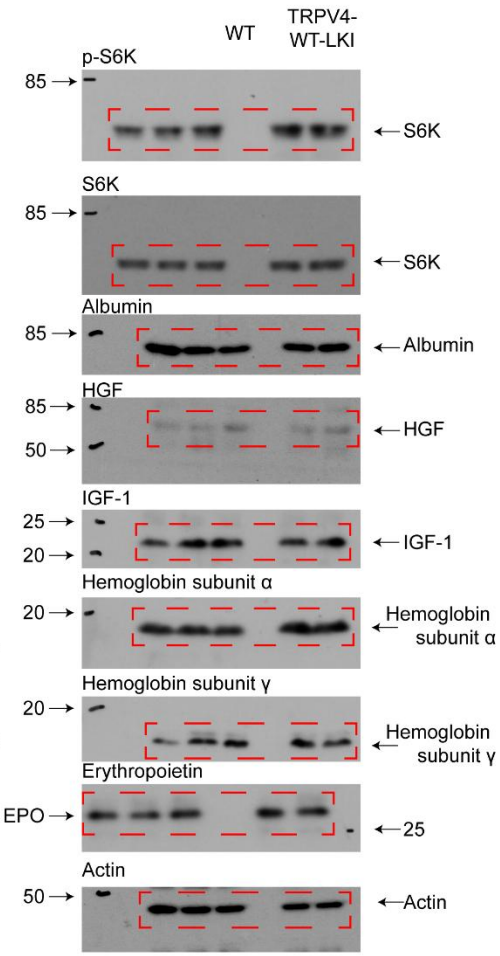

WT
TRPV4-
WT-LKI
p-S6K
85
S6K
S6K
85
S6K
Albumin
85
Albumin
HGF
85
50
HGF
IGF-1
25
20
IGF-1
Hemoglobin subunit α
20
Hemoglobin
subunit α
Hemoglobin subunit γ
20
Hemoglobin
subunit γ
Erythropoietin
EPO
25
Actin
50
Actin

Fig. S1A

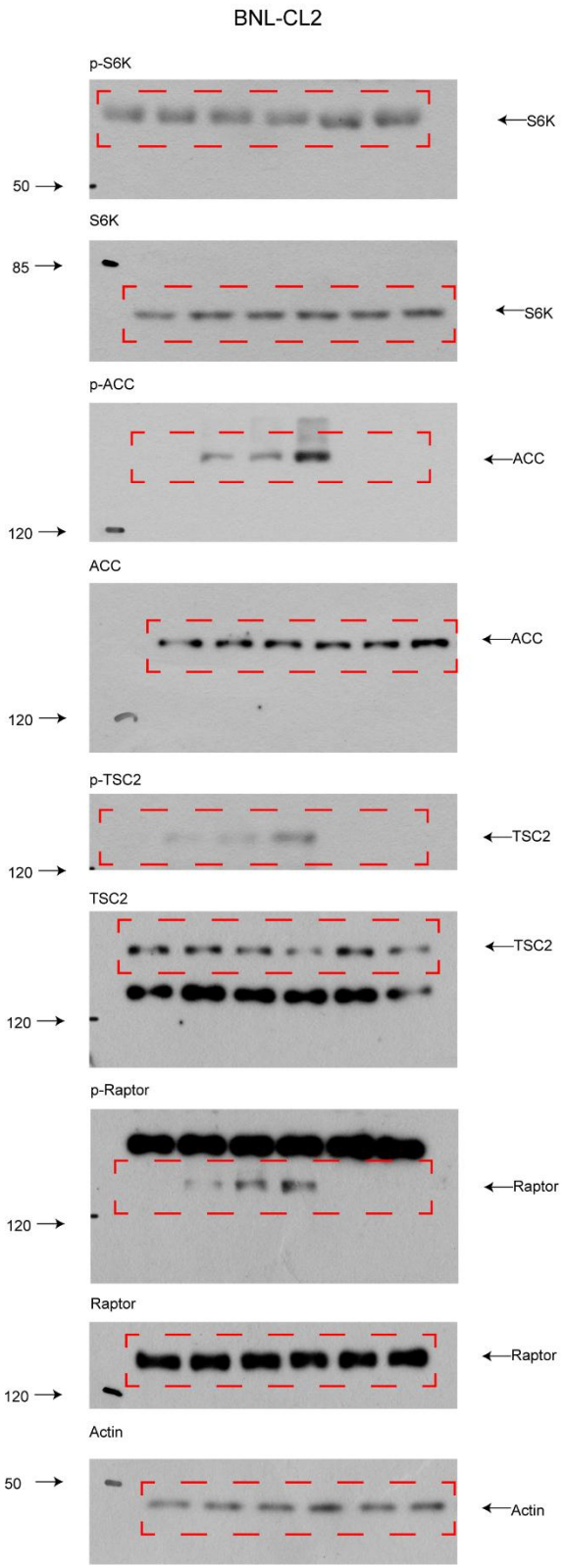


Fig. S1D

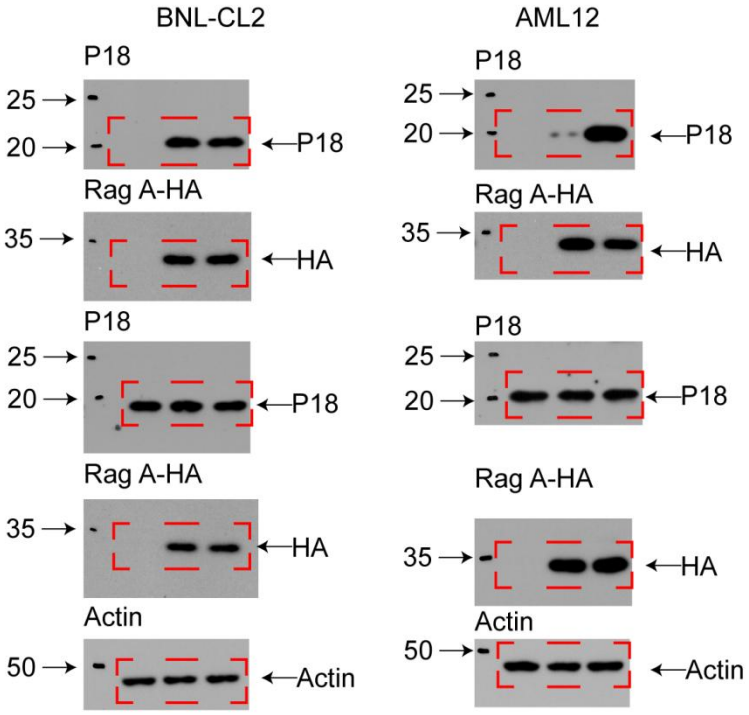

BNL-CL2
AML12
P18
25
20
P18
Rag A-HA
35
HA
P18
25
20
P18
Rag A-HA
35
HA
Actin
50
Actin

Fig. S4E

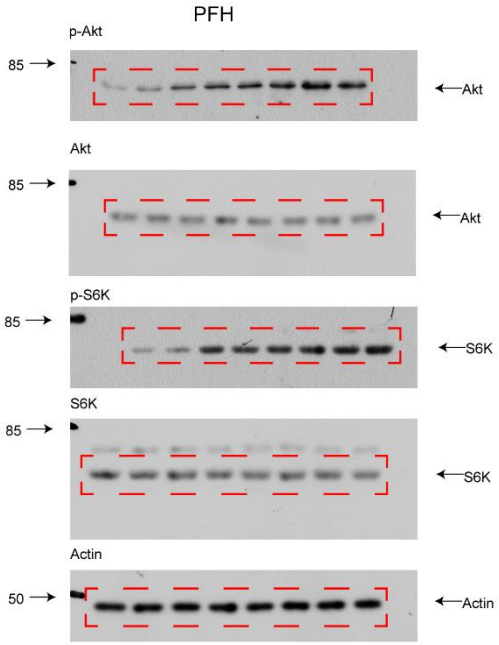


Fig. S4G

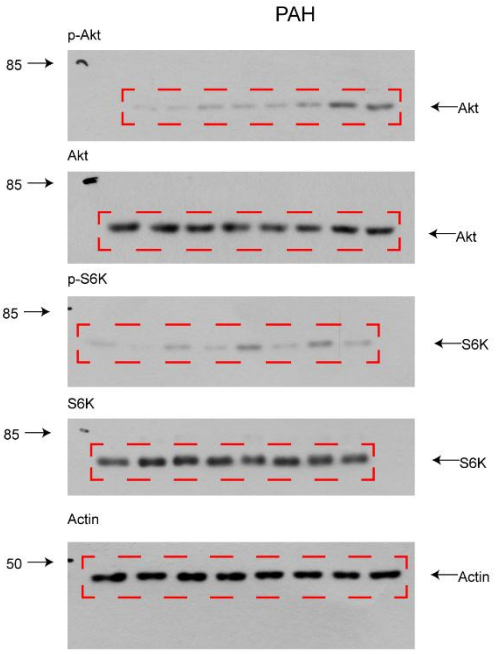


Fig. S4I

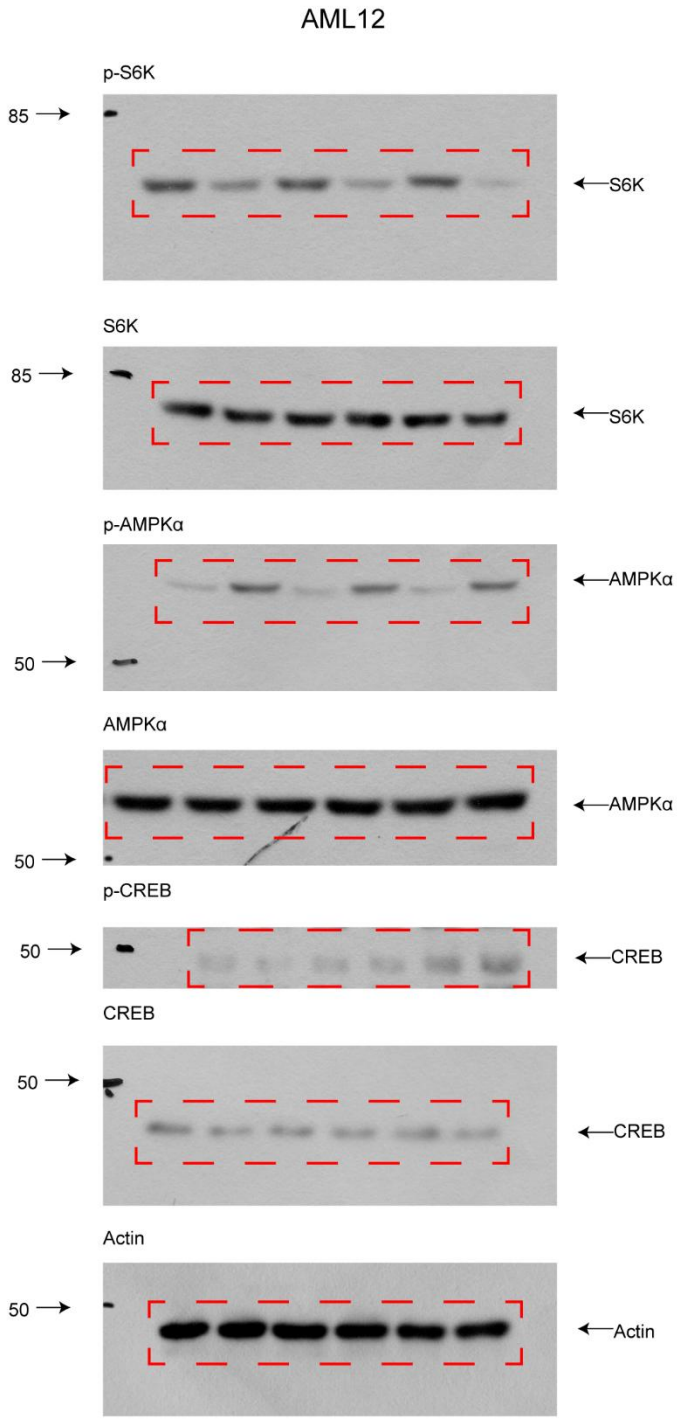

AML12
p-S6K
85
S6K
S6K
85
S6K
p-AMPKα
AMPKα
50
AMPKα
AMPKα
50
AMPKα
p-CREB
50
CREB
CREB
50
CREB
Actin
50
Actin

Fig. S9C

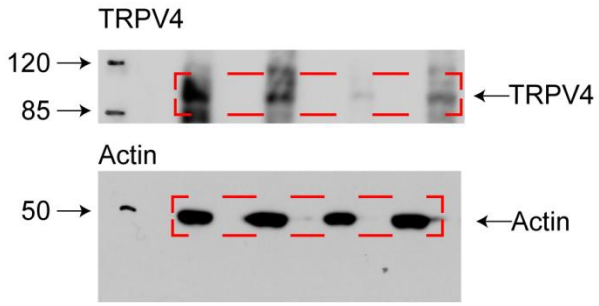


Fig. S10A

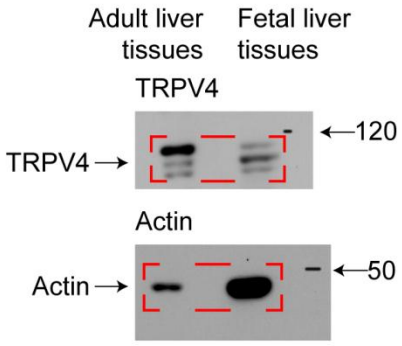


Fig. S10B

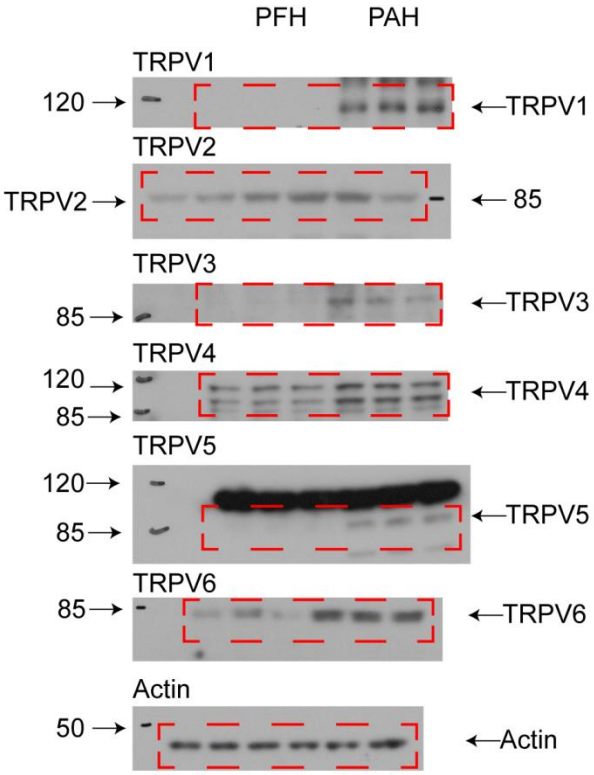


Fig. S11K

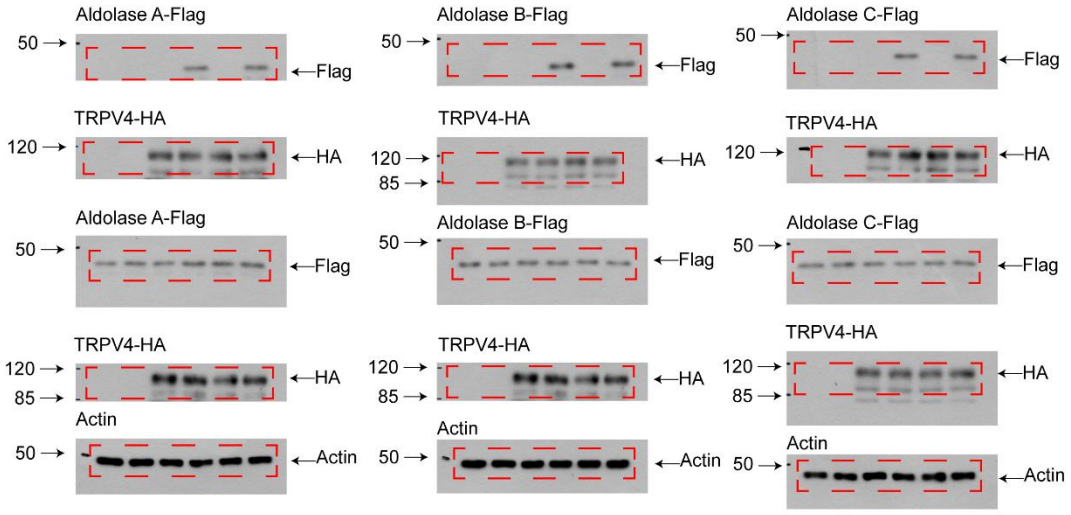

Aldolase A-Flag
50
Flag
Aldolase B-Flag
50
Flag
Aldolase C-Flag
50
Flag
TRPV4-HA
120
HA
TRPV4-HA
120
85
HA
TRPV4-HA
120
HA
Aldolase A-Flag
50
Flag
Aldolase B-Flag
50
Flag
Aldolase C-Flag
50
Flag
TRPV4-HA
120
85
HA
TRPV4-HA
120
85
HA
TRPV4-HA
120
85
HA
Actin
50
Actin
Actin
50
Actin
Actin
50
Actin

Fig. S12A, B

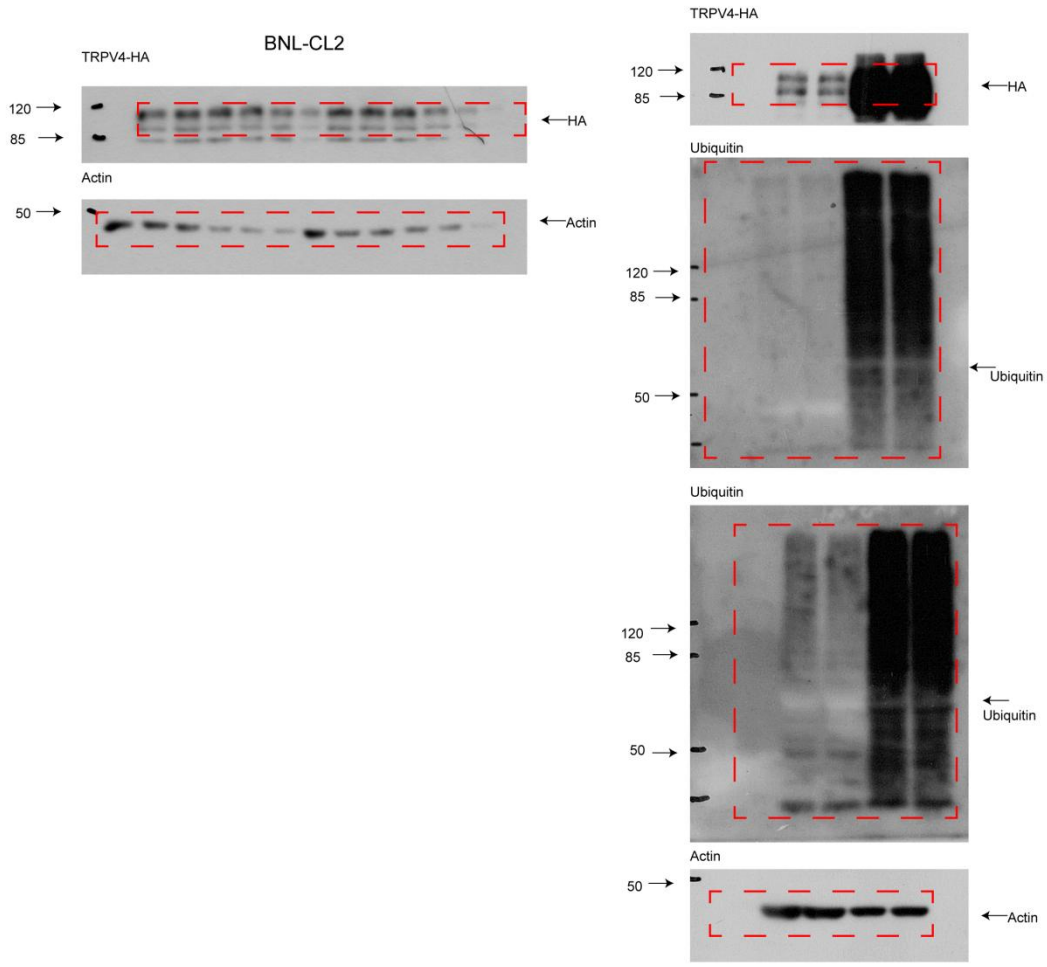

BNL-CL2
TRPV4-HA
120
85
HA
Actin
50
Actin
TRPV4-HA
120
85
HA
Ubiquitin
120
85
50
Ubiquitin
Ubiquitin
120
85
50
Ubiquitin
Actin
50
Actin

Fig. S12C

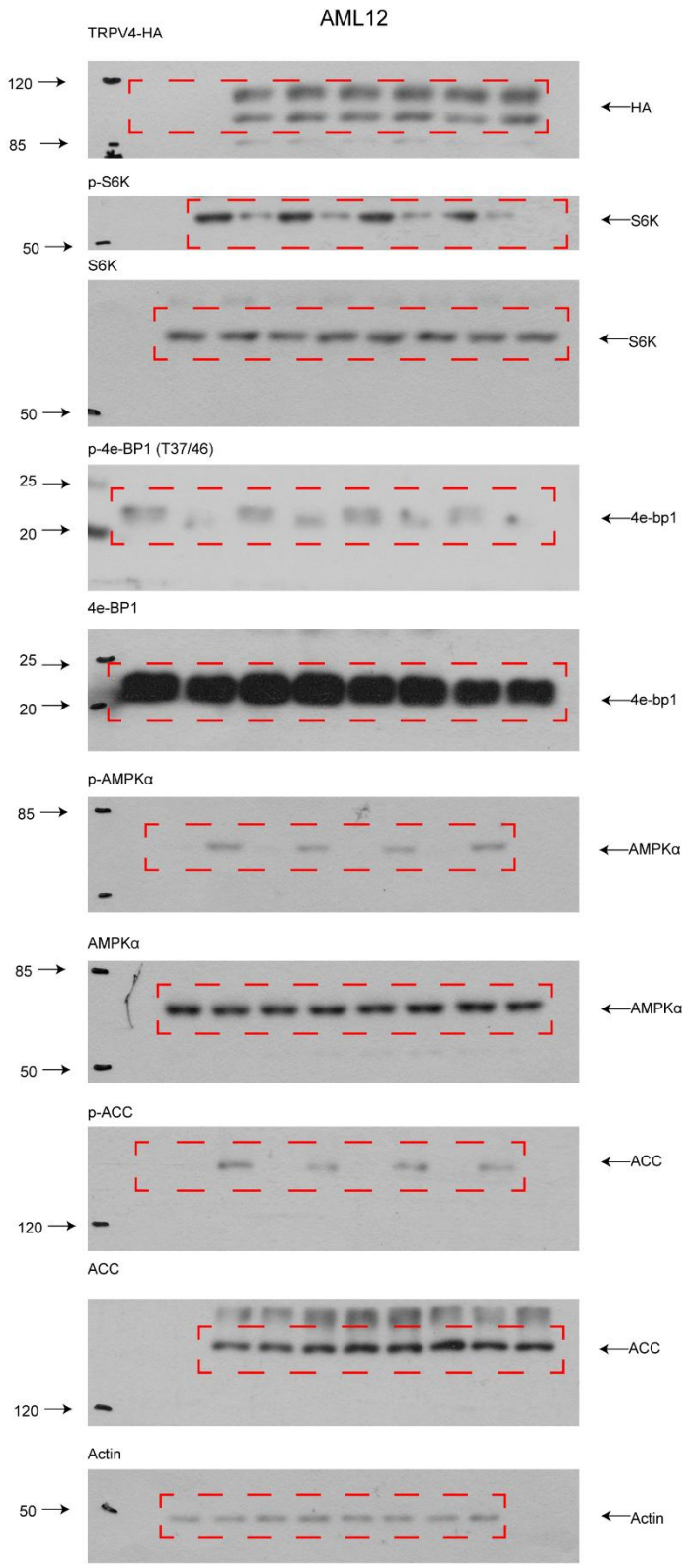

AML12
TRPV4-HA
120
85
HA
p-S6K
S6K
50
S6K
S6K
50
p-4e-BP1 (T37/46)
25
20
4e-bp1
4e-BP1
25
20
4e-bp1
p-AMPKα
85
AMPKα
AMPKα
85
AMPKα
50
p-ACC
ACC
120
ACC
ACC
120
Actin
50
Actin

Fig. S13A-D

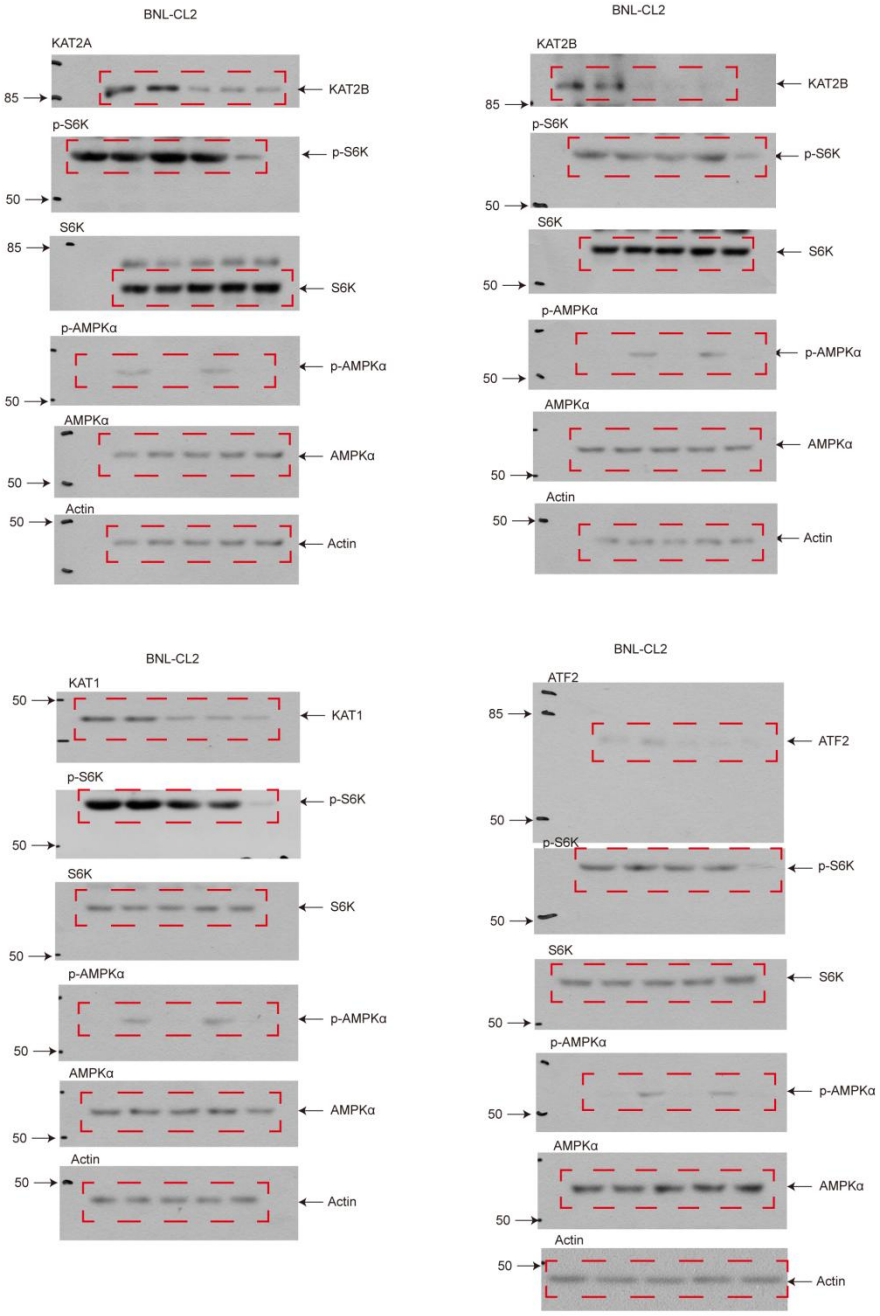

BNL-CL2
KAT2A
KAT2B
p-S6K
S6K
p-AMPKα
AMPKα
Actin
85
50
BNL-CL2
KAT2B
p-S6K
S6K
p-AMPKα
AMPKα
Actin
BNL-CL2
KAT1
p-S6K
S6K
p-AMPKα
AMPKα
Actin
BNL-CL2
ATF2
p-S6K
S6K
p-AMPKα
AMPKα
Actin

Fig. S13E-H

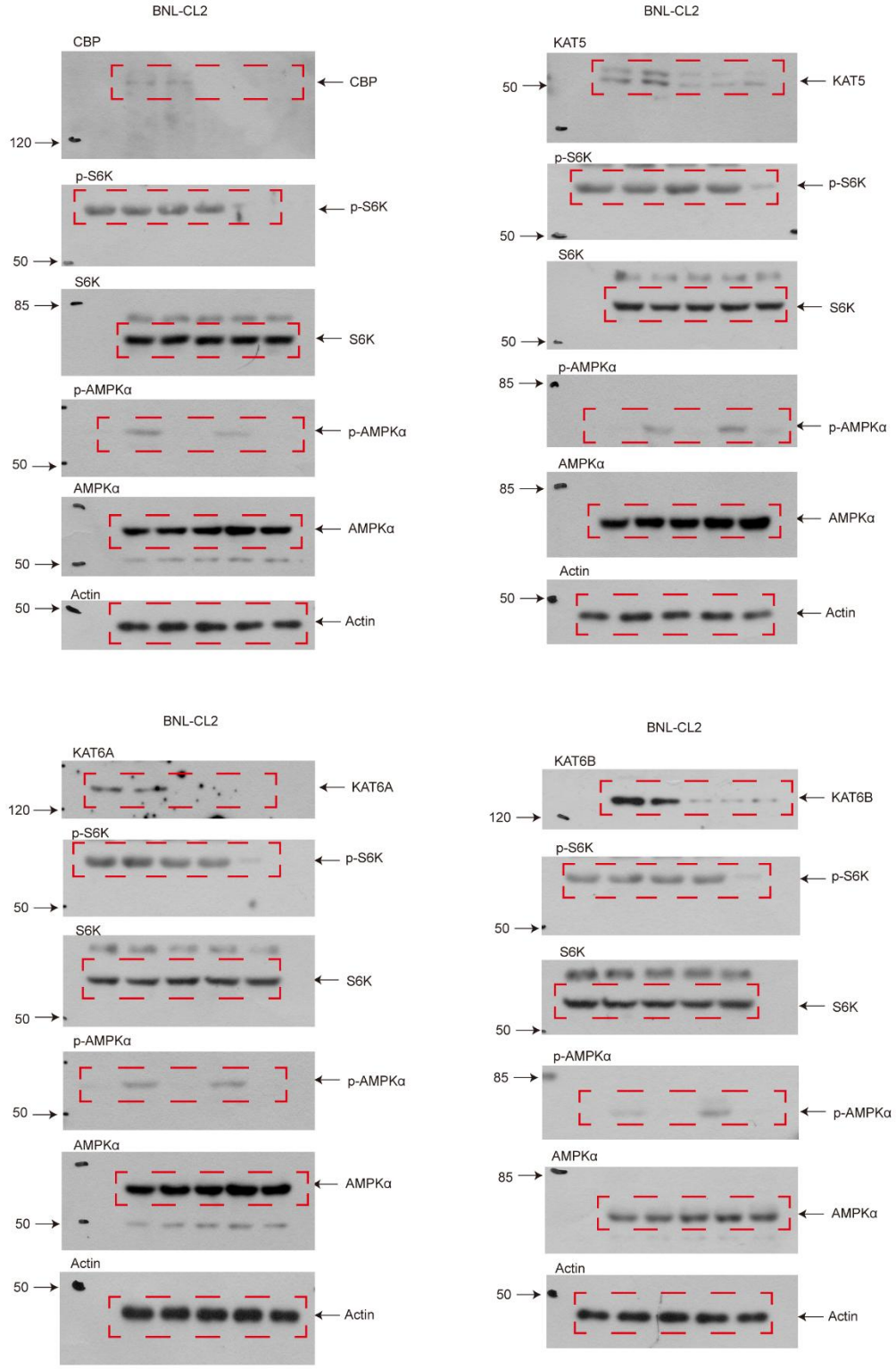

BNL-CL2
CBP
CBP
120
p-S6K
p-S6K
50
S6K
85
S6K
p-AMPKα
p-AMPKα
50
AMPKα
AMPKα
50
Actin
50
Actin
BNL-CL2
KAT5
50
KAT5
p-S6K
p-S6K
50
S6K
S6K
50
p-AMPKα
85
p-AMPKα
AMPKα
85
AMPKα
Actin
50
Actin
BNL-CL2
KAT6A
KAT6A
120
p-S6K
p-S6K
50
S6K
S6K
50
p-AMPKα
p-AMPKα
50
AMPKα
AMPKα
50
Actin
50
Actin
BNL-CL2
KAT6B
120
KAT6B
p-S6K
p-S6K
50
S6K
S6K
50
p-AMPKα
85
p-AMPKα
AMPKα
85
AMPKα
Actin
50
Actin

Fig. S13I-L

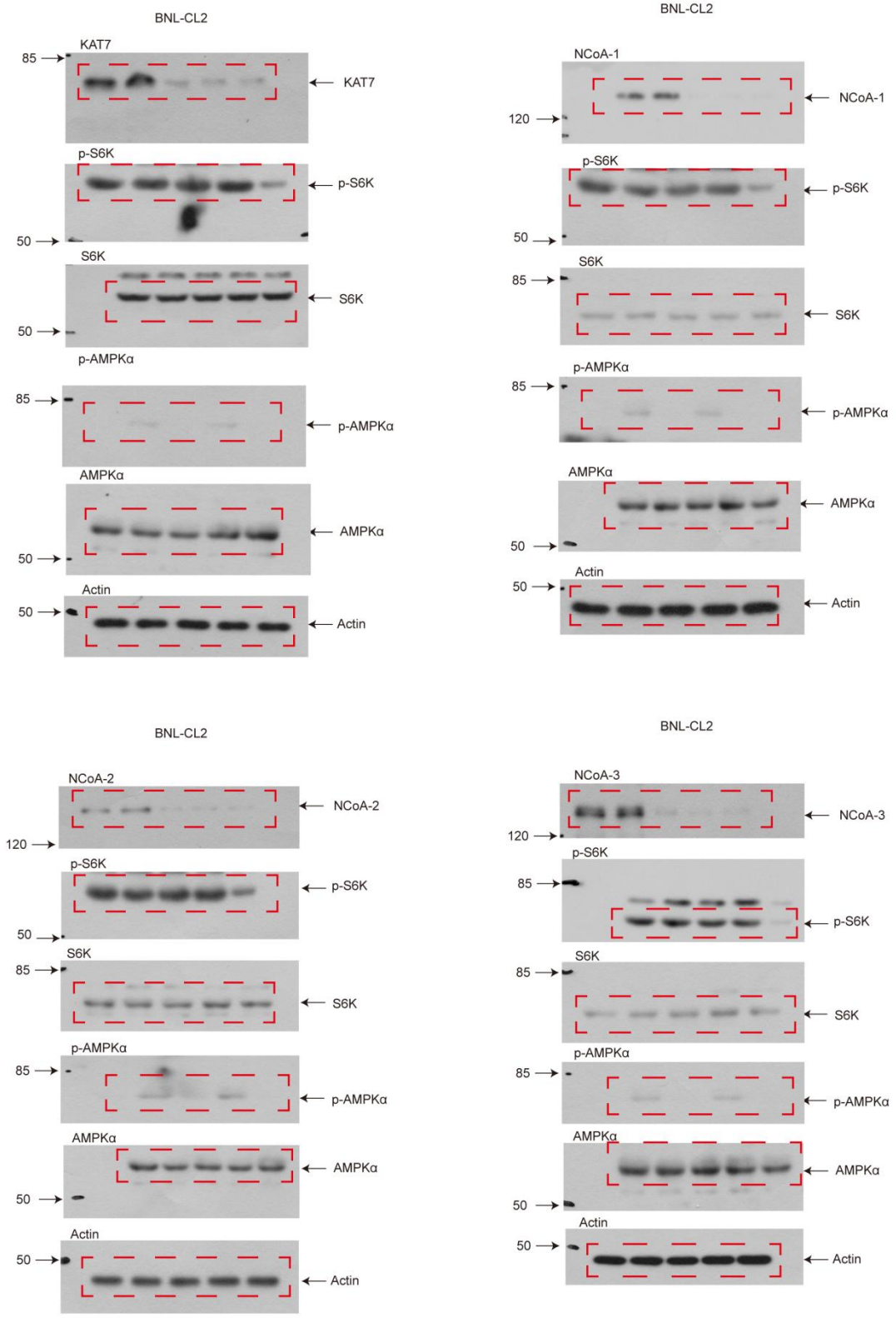
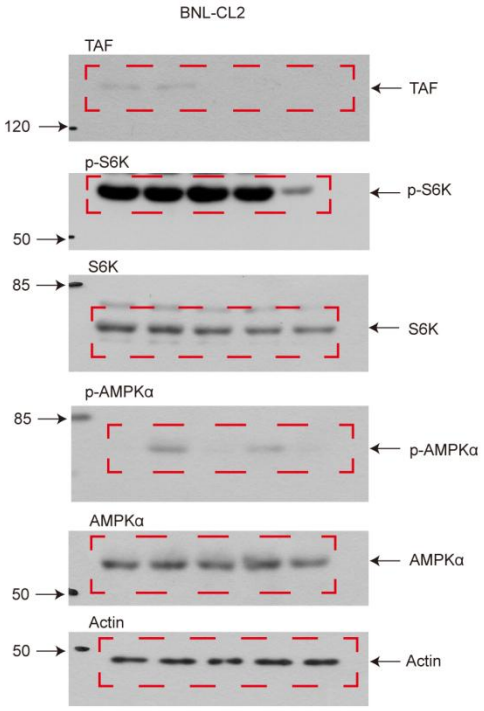
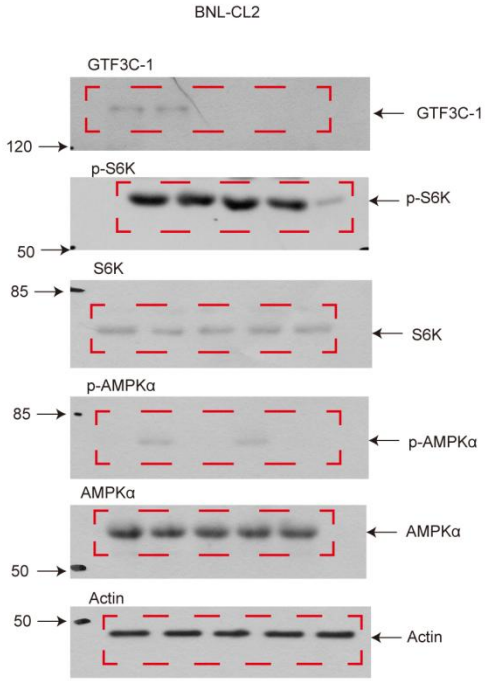

BNL-CL2
KAT7
85
KAT7
p-S6K
p-S6K
50
S6K
S6K
50
p-AMPKα
85
p-AMPKα
AMPKα
AMPKα
50
Actin
50
Actin
BNL-CL2
NCoA-1
120
NCoA-1
p-S6K
p-S6K
50
S6K
85
S6K
p-AMPKα
85
p-AMPKα
AMPKα
AMPKα
50
Actin
50
Actin
BNL-CL2
NCoA-2
120
NCoA-2
p-S6K
p-S6K
50
S6K
85
S6K
p-AMPKα
85
p-AMPKα
AMPKα
AMPKα
50
Actin
50
Actin
BNL-CL2
NCoA-3
120
NCoA-3
p-S6K
85
p-S6K
S6K
85
S6K
p-AMPKα
85
p-AMPKα
AMPKα
AMPKα
50
Actin
50
Actin

Fig. S13M-O


BNL-CL2
TAF
120
TAF
p-S6K
50
p-S6K
S6K
85
S6K
p-AMPKα
85
p-AMPKα
AMPKα
50
AMPKα
Actin
50
Actin


BNL-CL2
GTF3C-1
120
GTF3C-1
p-S6K
50
p-S6K
S6K
85
S6K
p-AMPKα
85
p-AMPKα
AMPKα
50
AMPKα
Actin
50
Actin

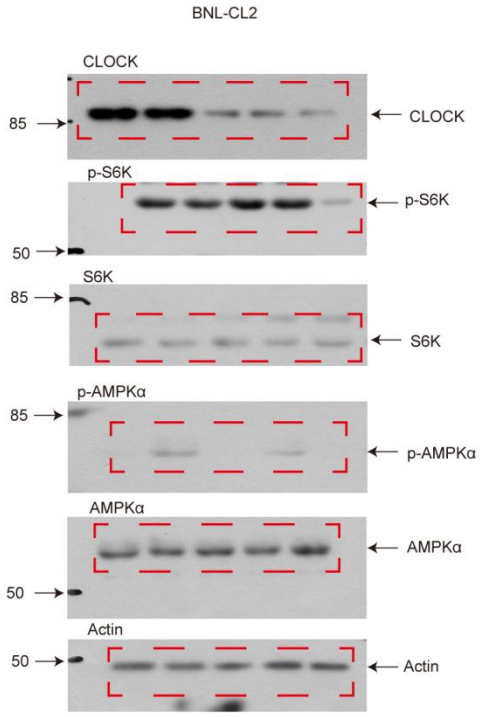

BNL-CL2
CLOCK
85
CLOCK
p-S6K
50
p-S6K
S6K
85
S6K
p-AMPKα
85
p-AMPKα
AMPKα
50
AMPKα
Actin
50
Actin

Fig. S14A-C

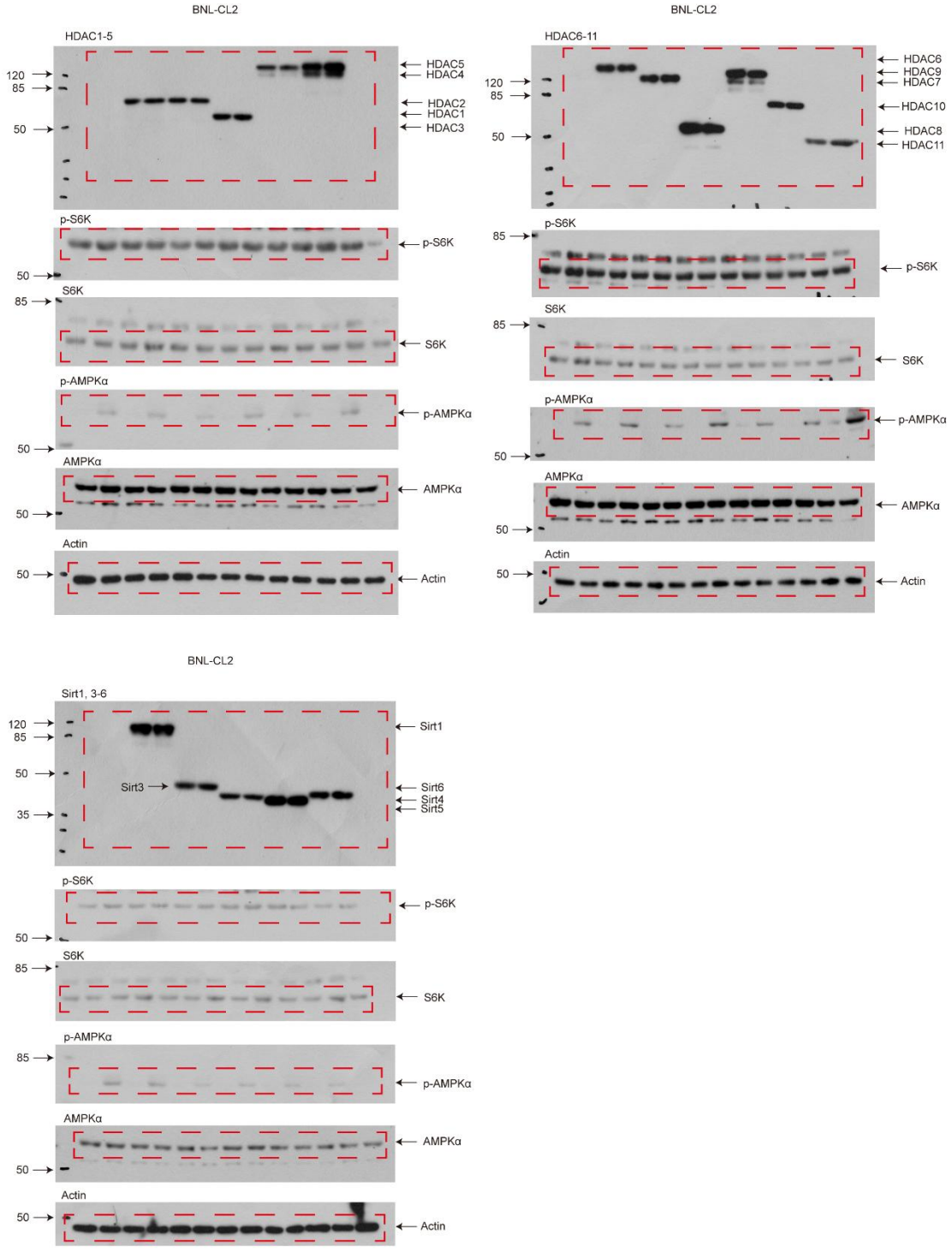

BNL-CL2
HDAC1-5
120
85
50
HDAC5
HDAC4
HDAC2
HDAC1
HDAC3
p-S6K
50
p-S6K
S6K
85
S6K
p-AMPKα
50
p-AMPKα
AMPKα
50
AMPKα
Actin
50
Actin
BNL-CL2
HDAC6-11
120
85
50
HDAC6
HDAC9
HDAC7
HDAC10
HDAC8
HDAC11
p-S6K
85
p-S6K
S6K
85
S6K
p-AMPKα
50
p-AMPKα
AMPKα
50
AMPKα
Actin
50
Actin
BNL-CL2
Sirt1, 3-6
120
85
50
35
Sirt3
Sirt1
Sirt6
Sirt4
Sirt5
p-S6K
50
p-S6K
S6K
85
S6K
p-AMPKα
85
p-AMPKα
AMPKα
50
AMPKα
Actin
50
Actin

Fig. S17D

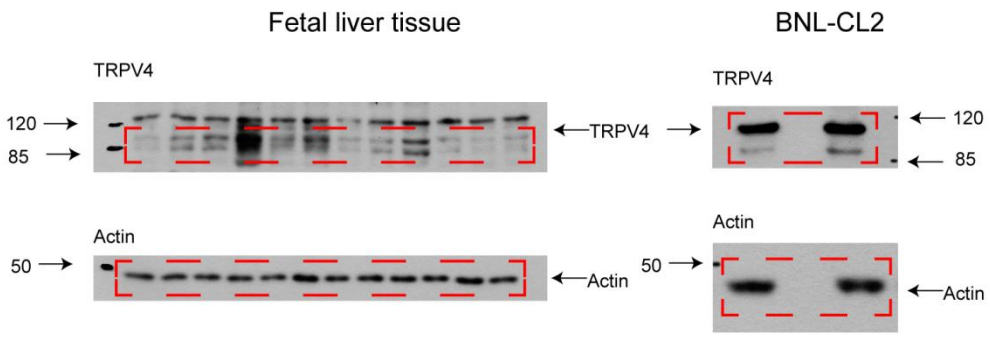

Fetal liver tissue
BNL-CL2
TRPV4
120
85
TRPV4
TRPV4
120
85
Actin
50
Actin
Actin
50
Actin

Fig. S19B

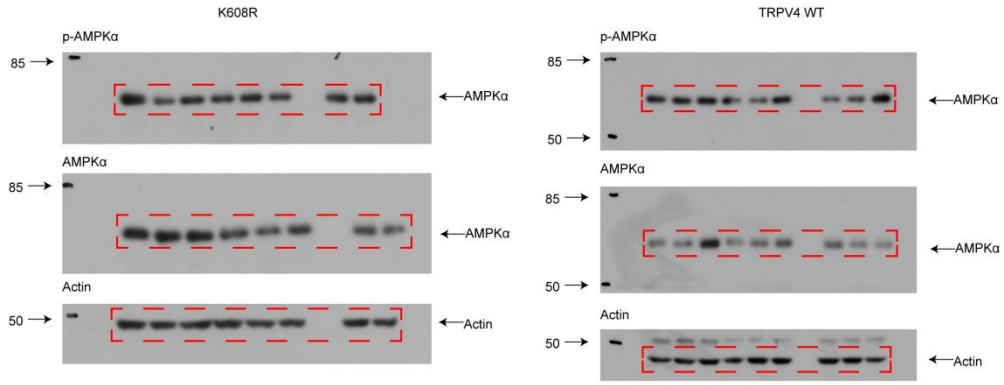

K608R
p-AMPKα
85
AMPKα
AMPKα
85
AMPKα
Actin
50
Actin
TRPV4 WT
p-AMPKα
85
50
AMPKα
AMPKα
85
50
AMPKα
Actin
50
Actin

Fig. S21F

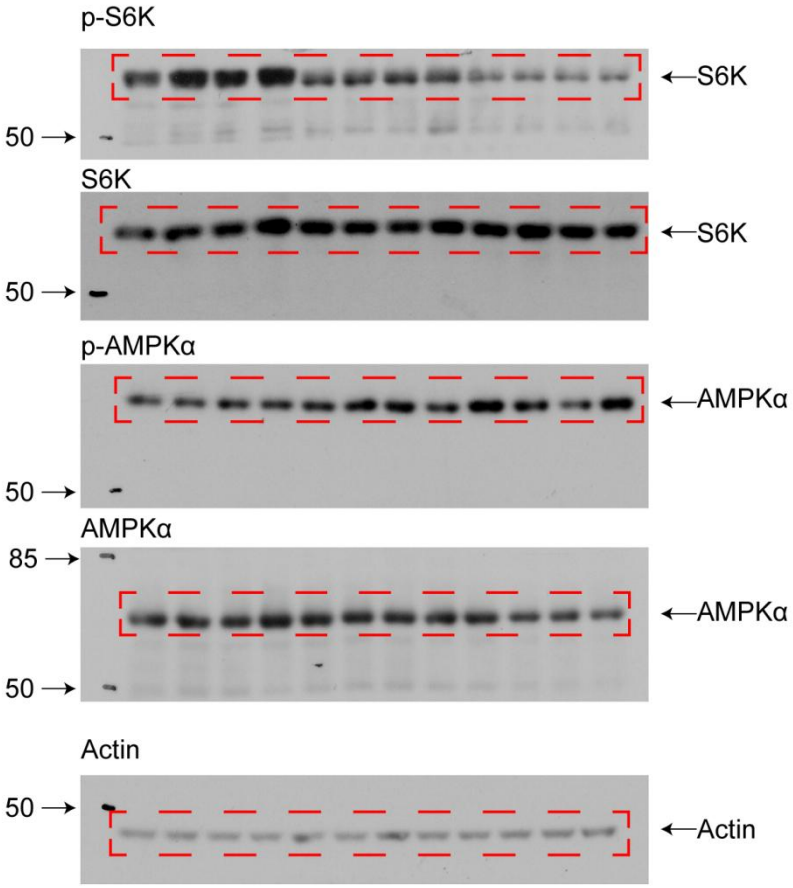
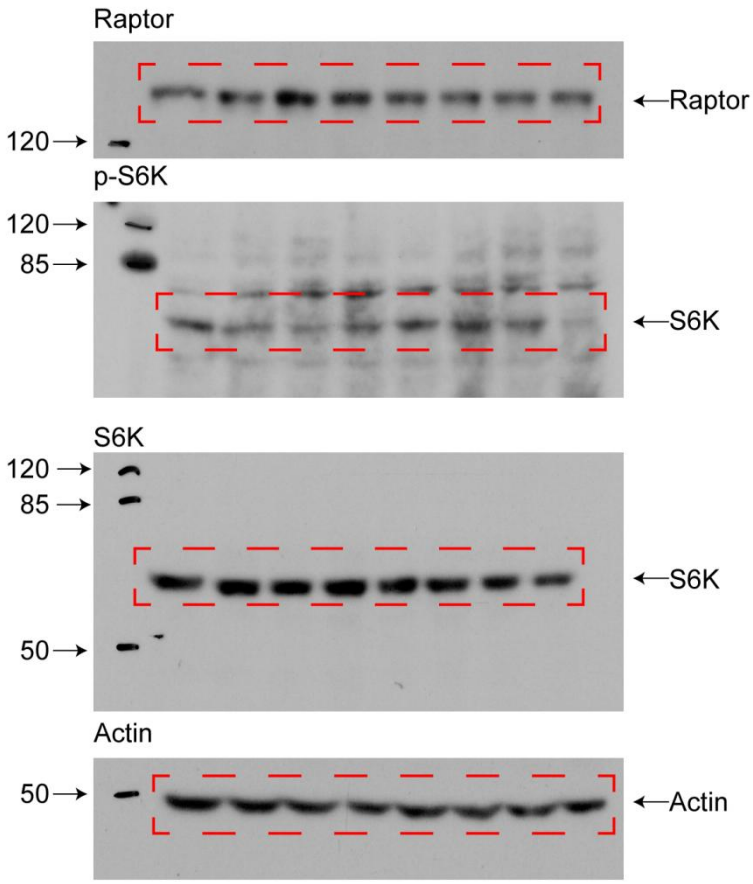

p-S6K
50
←S6K
S6K
50
←S6K
p-AMPKα
50
←AMPKα
AMPKα
85
50
←AMPKα
Actin
50
←Actin

Fig. S21G


Raptor
120
Raptor
p-S6K
120
85
S6K
S6K
120
85
50
S6K
Actin
50
Actin