

Supplementary Material to:

Continued low-frequency Western diet consumption promotes sustained insulin resistance and non-alcoholic fat liver disease in C57BL/6J mice

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Keywords: Ultra-processed food; type 2 diabetes; intermittent hypercaloric diet; NAFLD; inflammation

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Table S1: Chow and High-Fat High-Sucrose (HFHS) diets composition

Diet	Chow			HFHS		
Ingredients	g/100g	Energy provided (kcal/100g)	Energy (%)	g/100g	Energy provided (kcal/100g)	Energy (%)
Casein	20.00	80.0	21.5	20.00	80.0	14.7
L-cystine	0.18	0.7	0.2	0.18	0.7	0.1
Sucrose	10.00	40.0	10.8	28.69	107.6	19.8
Alpha-cellulose	5.00			5.00		
Starch	40.00	160.0	43.0			
Dextrinized Starch	13.09	53.4	14.3			
Mineral mix (AIN-93)	3.50			4.90		
Vitamin mix (AIN-93)	1.00			1.40		
Lard	-			19.80	178.2	32.7
Soy oil	7.00	38.1	10.2	19.80	178.2	32.7
Choline bitartrate	0.20			0.20		
Tert-butylhydrotoluene (BHT)	0.03			0.03		
Total	100.00	372.2	100	100.00	544.7	100

Table S2. Antibodies used

Antibody	Brand	Catalog #	Dilution
ACLY	Abcam ^{&}	40793	1:1000
Akt	Cell Signaling [*]	9272	1:1000
Anti-mouse	Jackson Labs [#]	115-035-146	1:10000
Anti-rabbit	Jackson Labs	115-035-144	1:20000
ATF6	SCBT [@]	22799	1:1000
CHOP	Cusabio [§]	CSB-PA001639	1:1000
IRE1 α	Cell Signaling	3294	1:1000
Phospho-ACLY (S455)	Abcam	46796	1:1000
Phospho-Akt (S473)	Cell Signaling	9271	1:1000
Phospho-Akt (T308)	Cell Signaling	9275	1:1000
Phospho-STAT3(Y705)	Cell Signaling	9131	1:1000
PKC δ	Cell Signaling	2058	1:1000
SIRT1	SCBT	15404	1:1000
STAT3	Cell Signaling	9138	1:1000
β -actin	Cell Signaling	4970	1:1000

* Cell signaling Technology, Danvers, MA, USA

& Abcam, Cambridge, UK

Jackson ImmunoResearch Labs, West Grove, PA, USA

@ Santa Cruz Biotechnology, Santa Cruz, CA, USA

§ Cusabio Technology, Wuhan Shi, China

Table S3. Oligo pairs.

Gene	Forward sequence	Reverse sequence	Amplicon length (bp)	Efficiency (%)
<i>Adgre1</i>	CCGTCAGGTACGGGATGAAT	AGAAGTCTGGGAATGGGAGC	73	97
<i>Arg1</i>	CCTCGAGGAGGGGTAGAGAAA	GGTCTCTCACGTCATACTCTGTTT	91	93
<i>Cd36</i>	AGAATTCTCAGCTGCTCCGC	CACATTTTCAGAAGGCAGCAAC	137	109
<i>Cpt1a</i>	TGGACCCAAATTGCAGTGGT	CTCCCACCAGTCACTCACATAA	73	97
<i>Egr2</i>	GTGGCGGGAGATGGCATGAT	TCGGATACGGGAGATCCAGG	70	97
<i>Fasn</i>	CCATGGCAGCTGTTGGTTTG	GTGTCCTCAGAGTTGTGGCA	88	98
<i>Fpr2</i>	CCAGTGATTCAAGCACCAAGTT	TCACAGACTTCATGGGGCCTT	71	99
<i>IL1b</i>	AGCTTCAGGCAGGCAGTATC	ACGGGAAAGACACAGGTAGC	70	102
<i>Ppargc1a</i>	CTFTGGAAGTGCAGGCCTAA	CAAGAGGGCTTCAGCTTTGG	96	99
<i>Ppara</i>	GCAACAACCCGCCTTTTGTC	TTGGCCACAAGCGTCTTCTC	70	99
<i>Pparg</i>	GCCTATGAGCACTTCACAAGAAAT	GCTGATTCCGAAGTTGGTGG	76	97
<i>Rpl7</i>	GAAGCTCATCTATGAGAAGGC	AAGACGAAGGAGCTGCAGAAC	202	105
<i>Srebf1</i>	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT	70	101
<i>Tnfa</i>	TGAGAAGTTCCCAAATGGCCT	CCACTTGGTGGTTTGTGAGTG	74	101
<i>Xbp1</i>	AAGAACACGCTTGGGAATGG	ACTCCCCTTGGCCTCCAC	67	99
<i>Xbp1s</i>	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA	65	102



Figure S1. Schematic representation of the animal protocol. The three different animal groups, named Chow, HFHS and HFHSw received different dietetic treatment for 12 weeks. The Chow group continuously received regular chow diet. The HFHS group continuously received the High-Fat High-Sucrose (HFHS) diet, while the HFHSw group received the HFHS diet once-a-week and the regular chow diet for the rest of the week.

Uncropped Gels

Figure 3A Originals cropped membranes

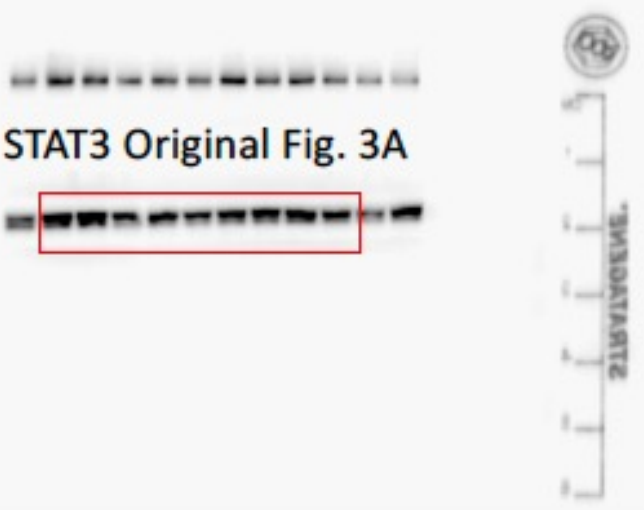
Sirt-1 Original Fig. 3A



P-STAT3 Original Fig. 3A



STAT3 Original Fig. 3A



β -actina

Original Figure 3A

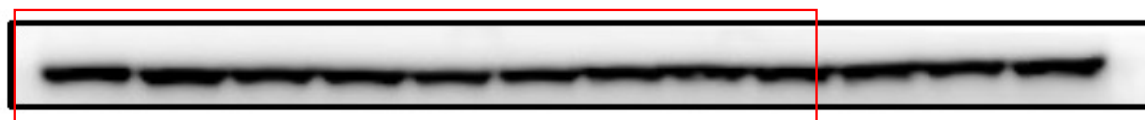


Figure 3A

PKCd Original

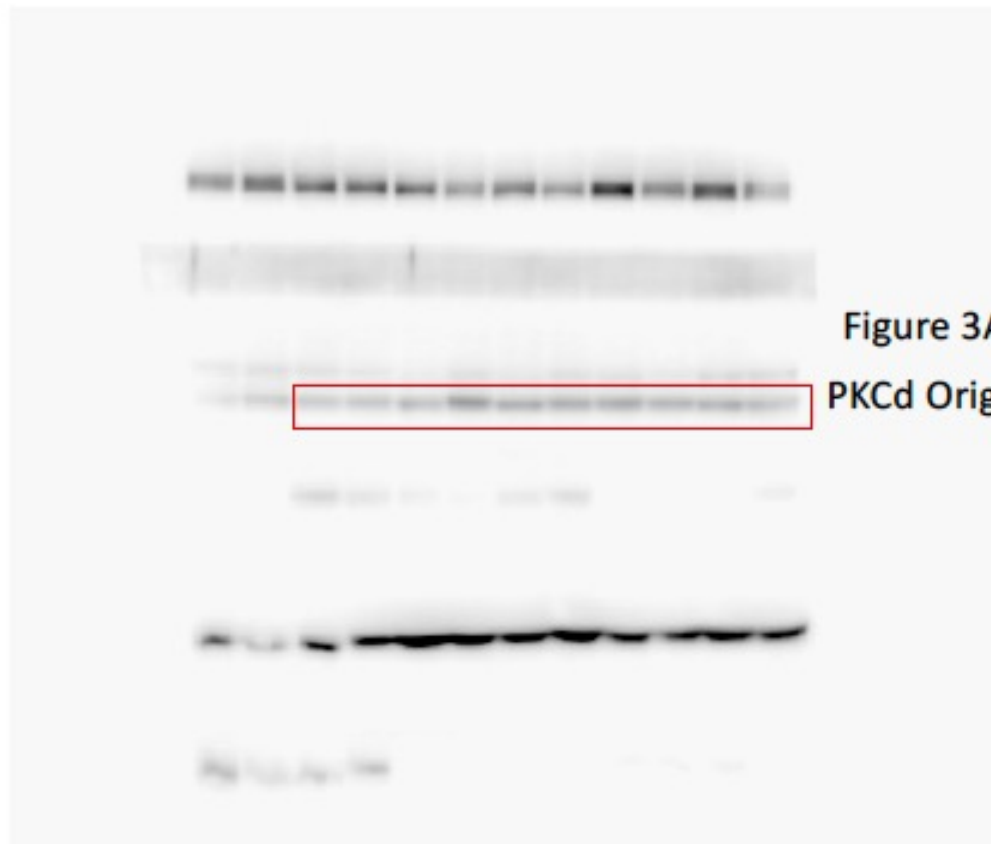


Figure 3F Originals cropped membranes

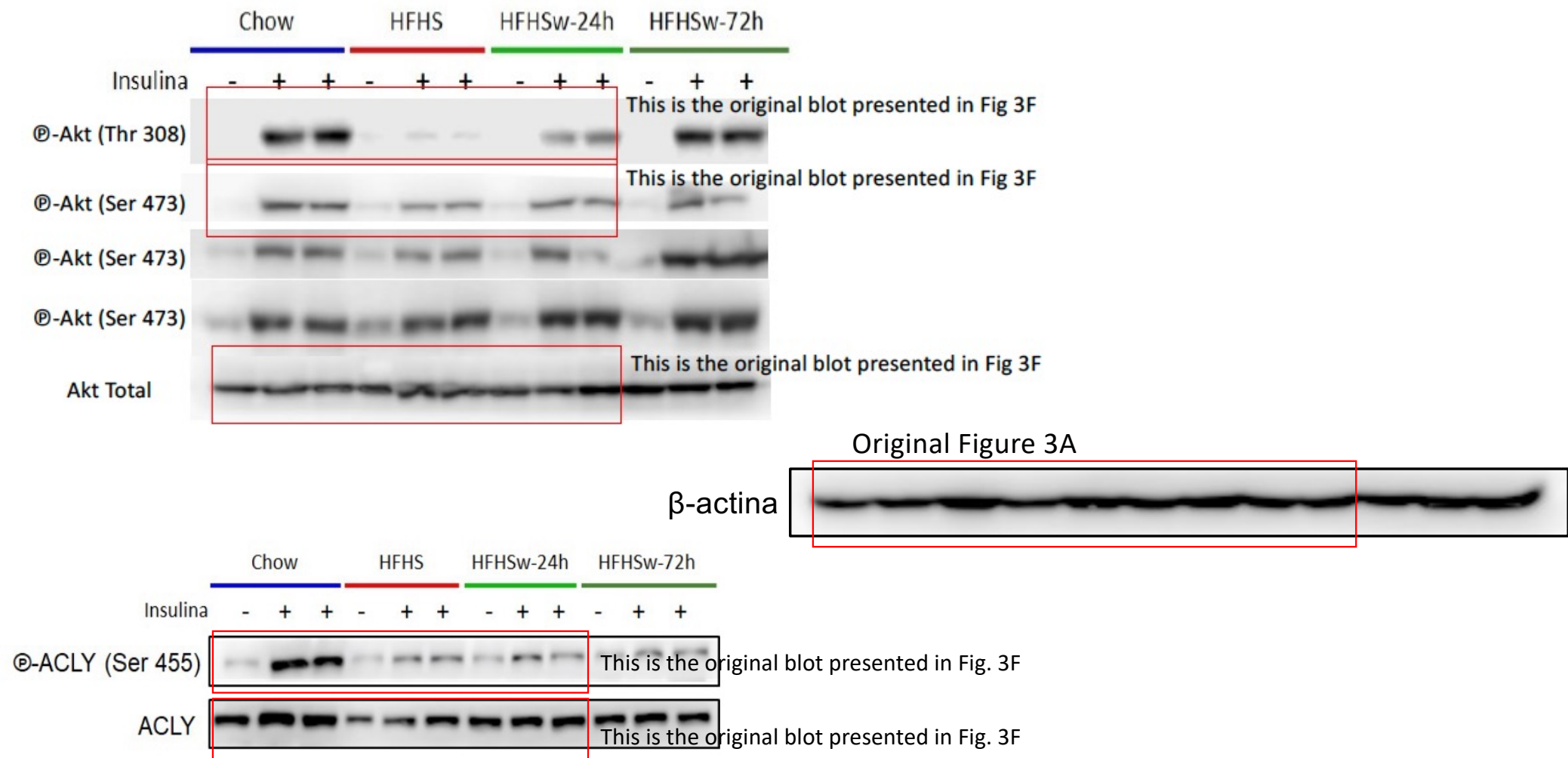


Figure 4A Originals cropped membranes



This is the original CHOP blot presented in Fig. 4A

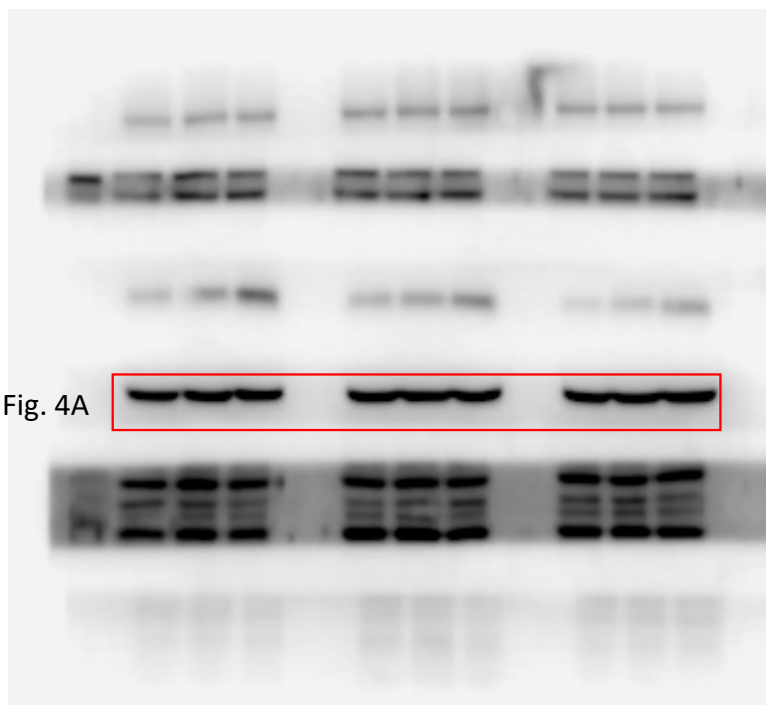


This is the original ATF6 blot presented in Fig. 4A



This is the original IRE1 blot presented in Fig. 4A

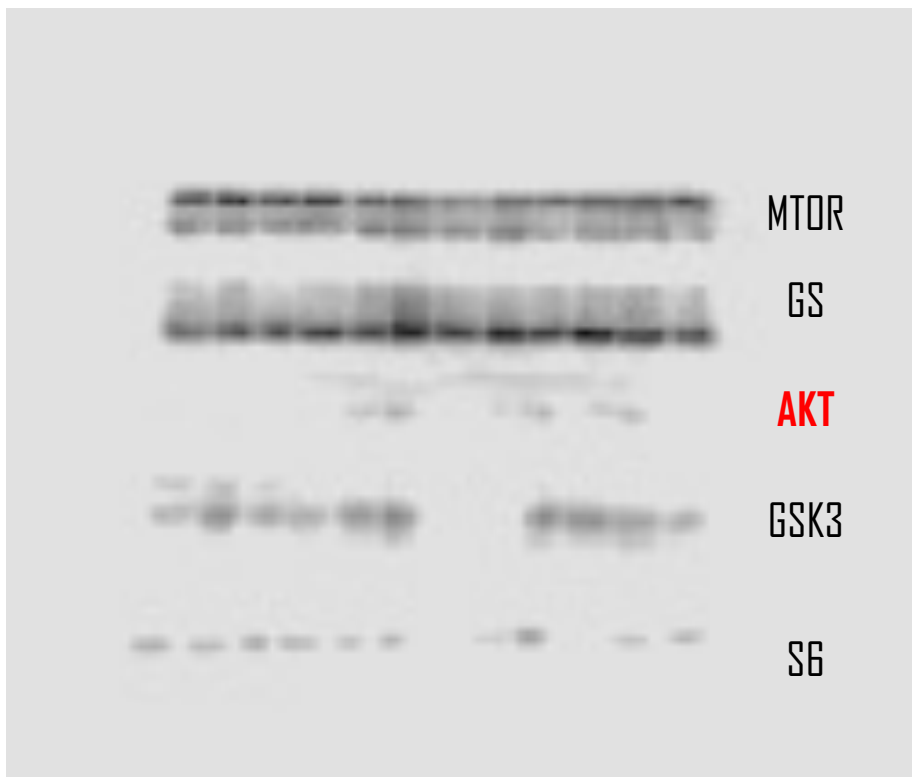
This is the original Actib blot presented in Fig. 4A



2018

13/03/2018

Gel 1



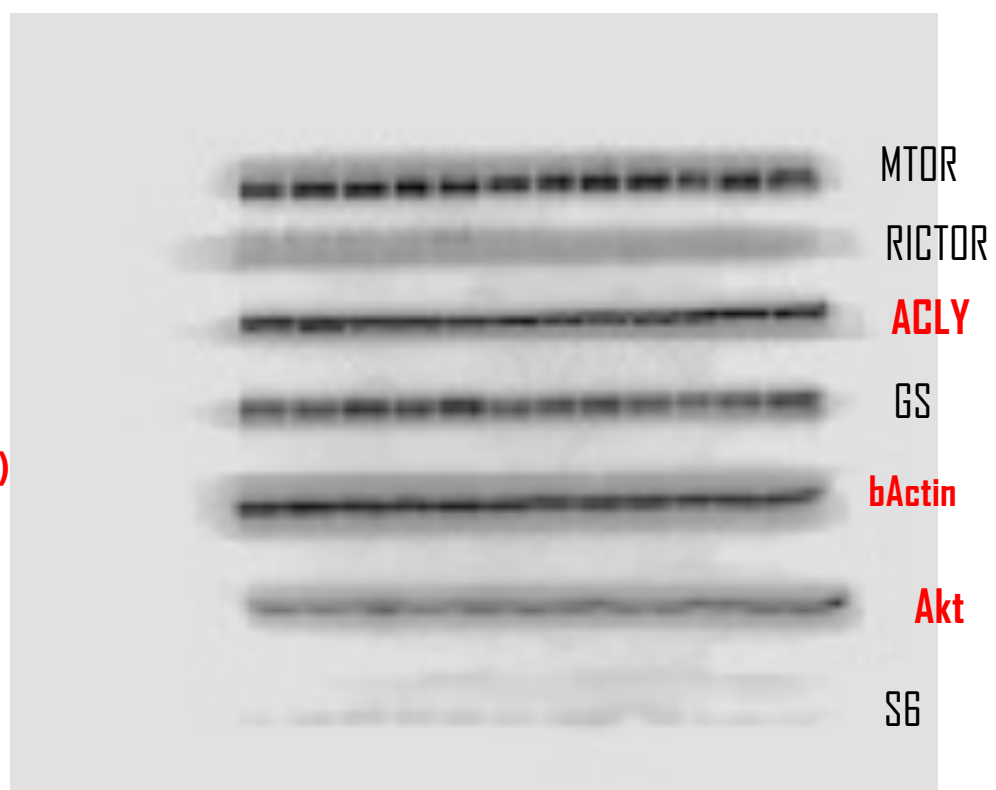
2018

03/09/2018

Gel 3



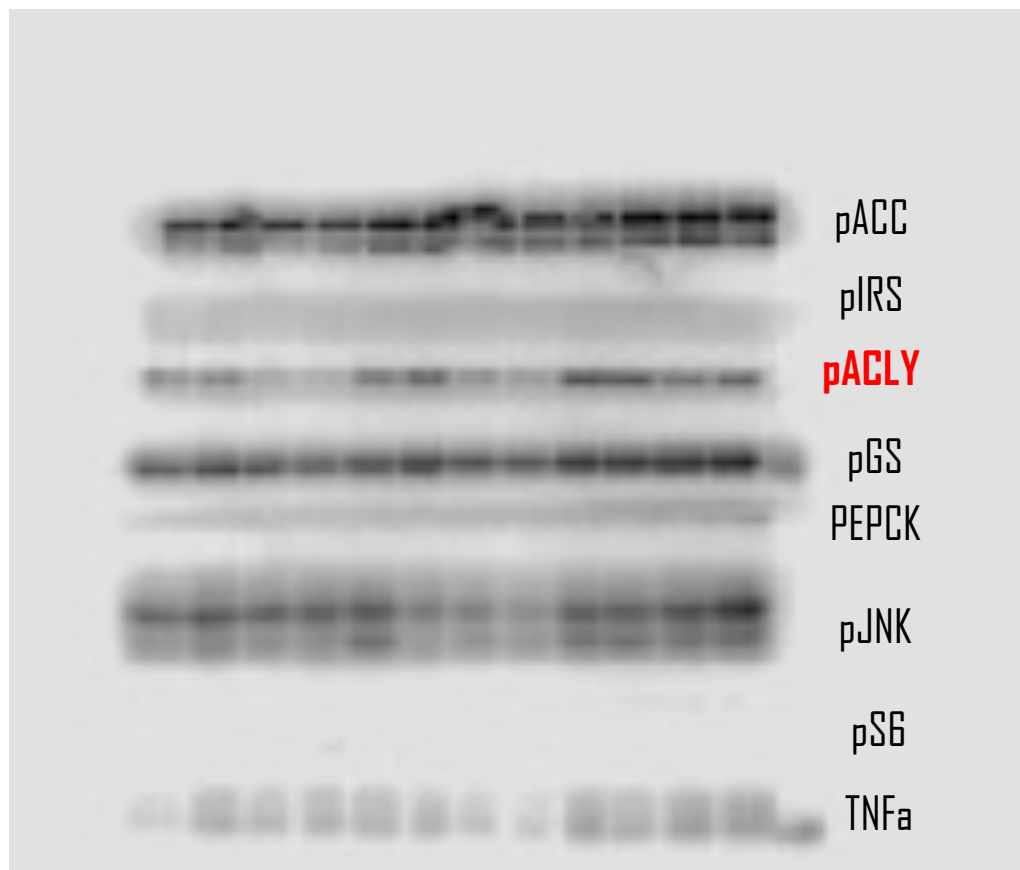
Gel 4



2018

10/04/2018

Gel 1



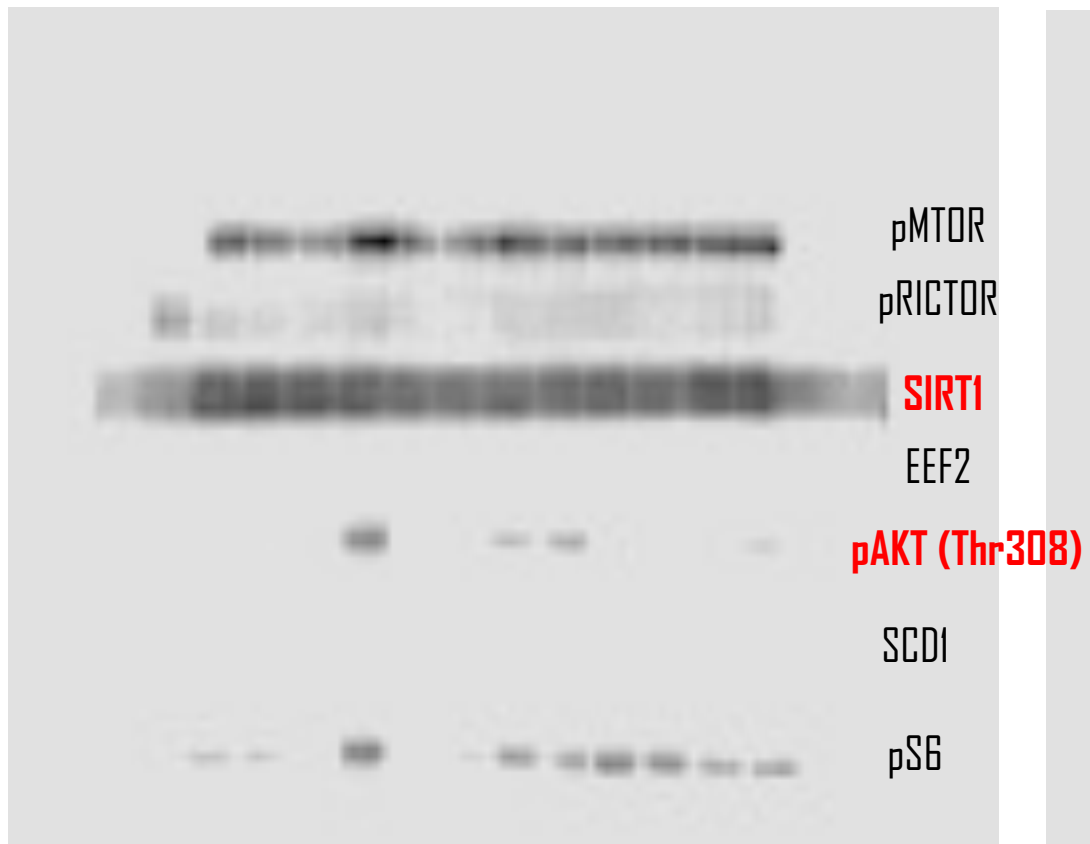
Gel 2



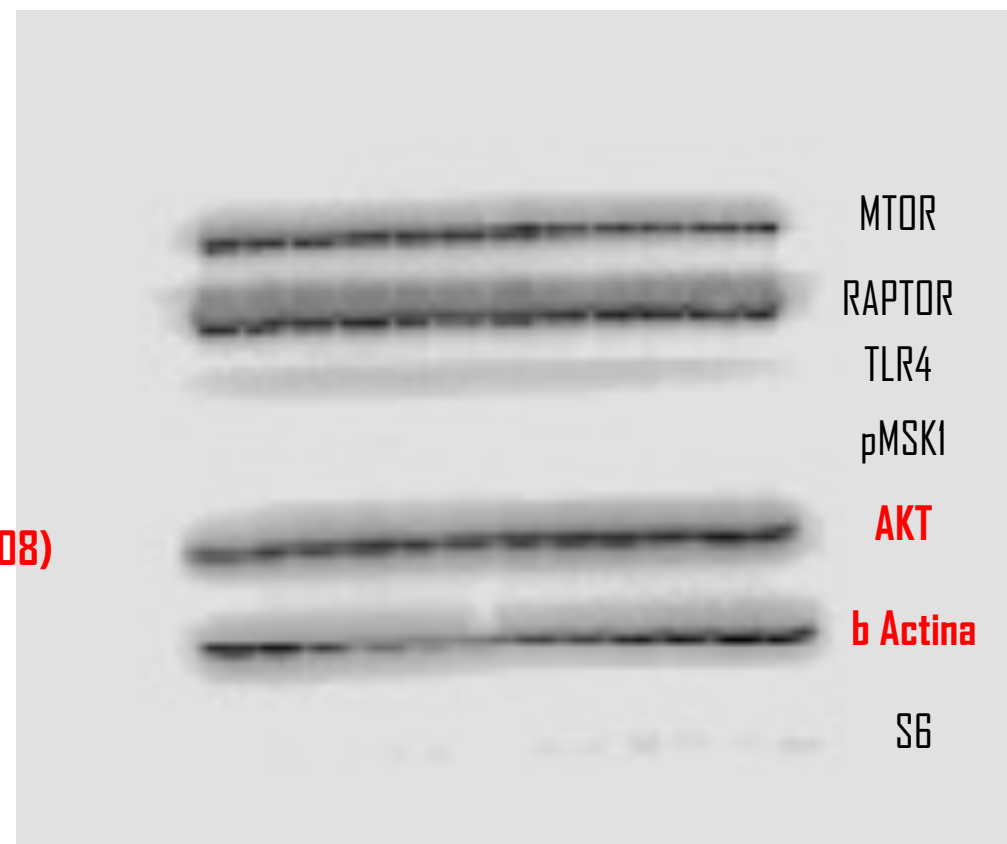
2018

10/04/2018

Gel 3



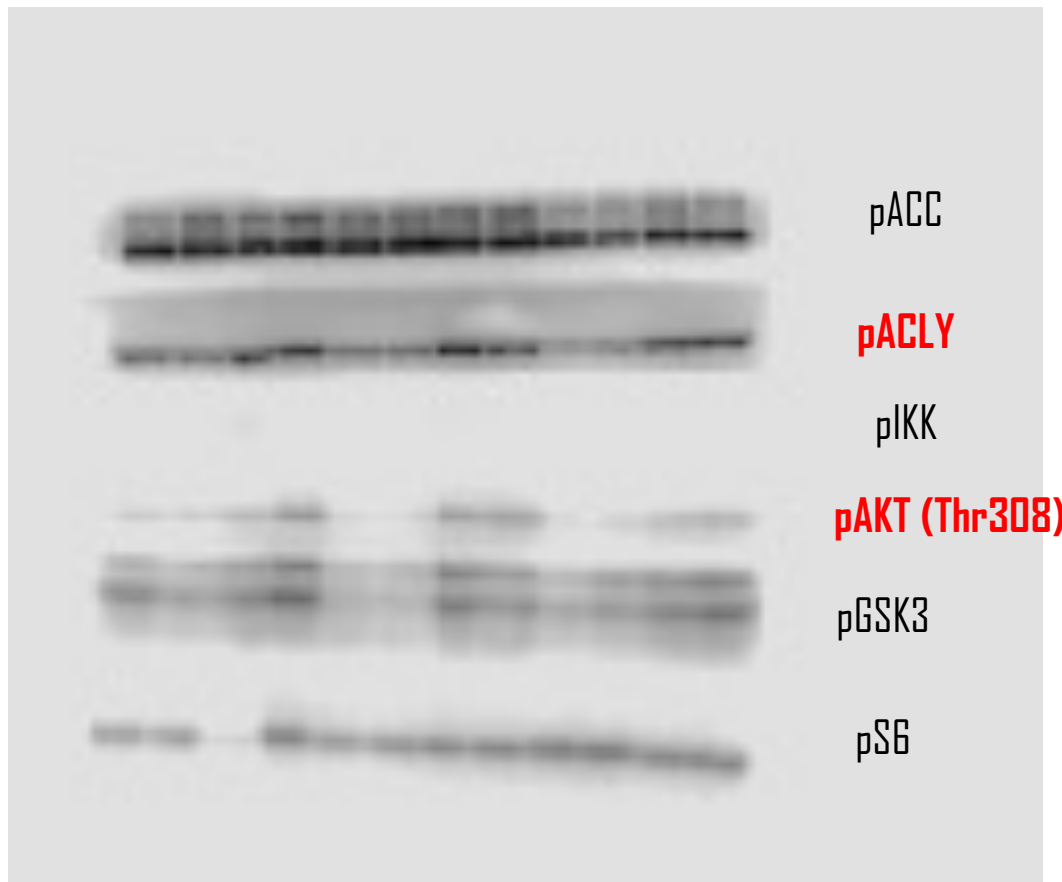
Gel 4



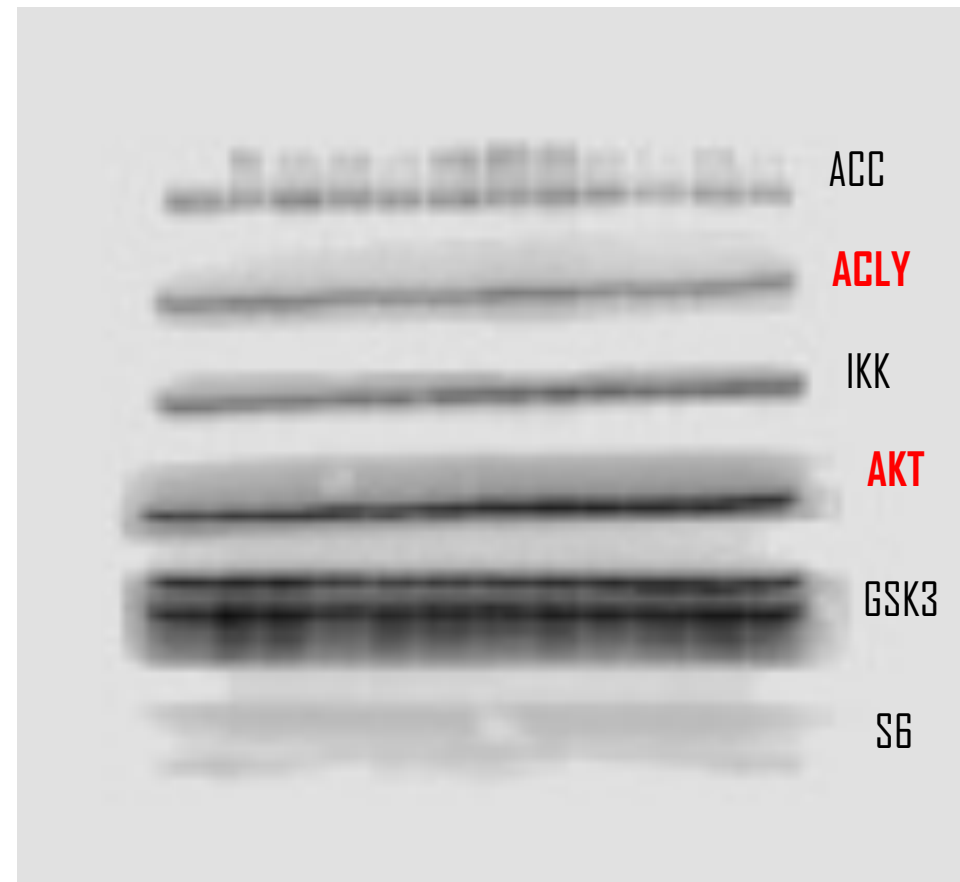
2018

16/04/2018

Gel 1



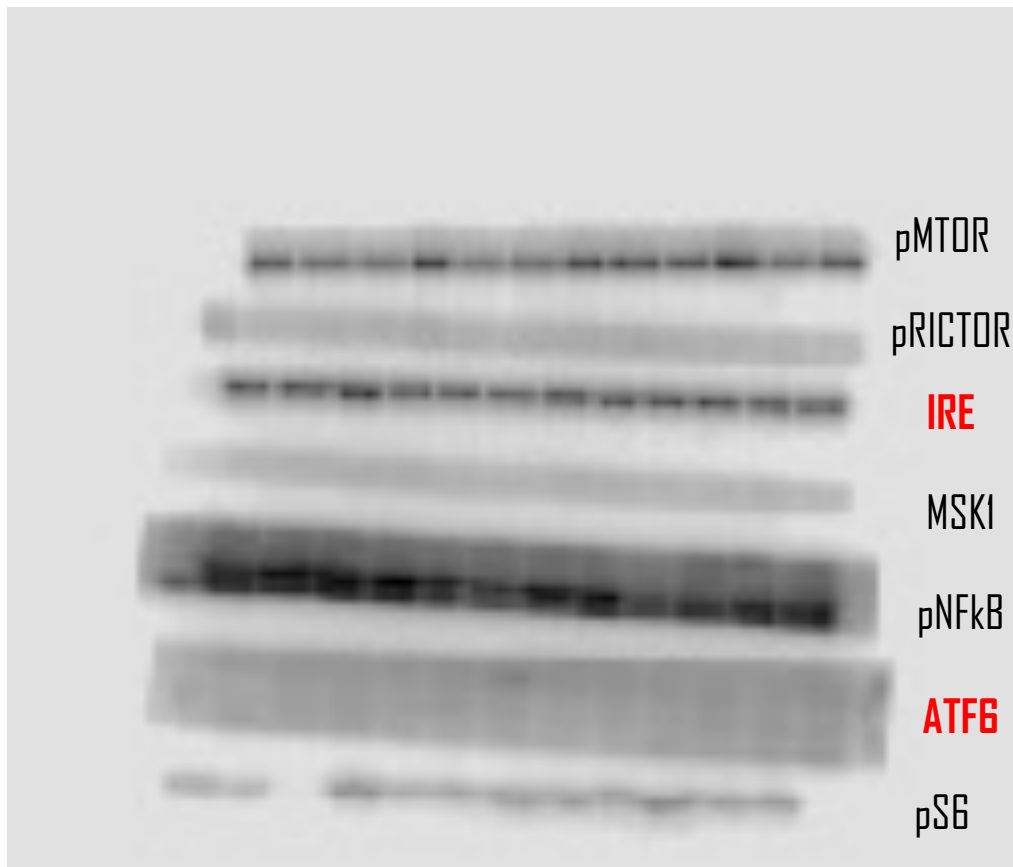
Gel 2



2018

17/04/2018

Gel 5



Gel 6



2018

18/04/2018

Gel 1

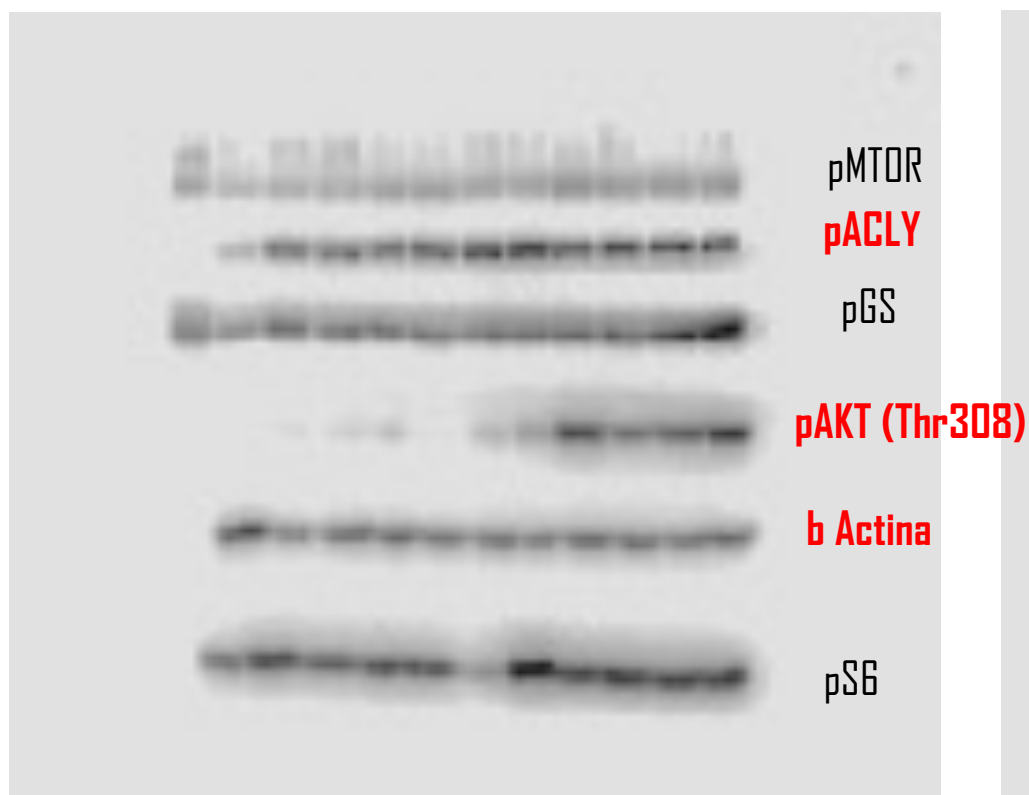


2018

12/06/2018

Gel 1

Gel 2



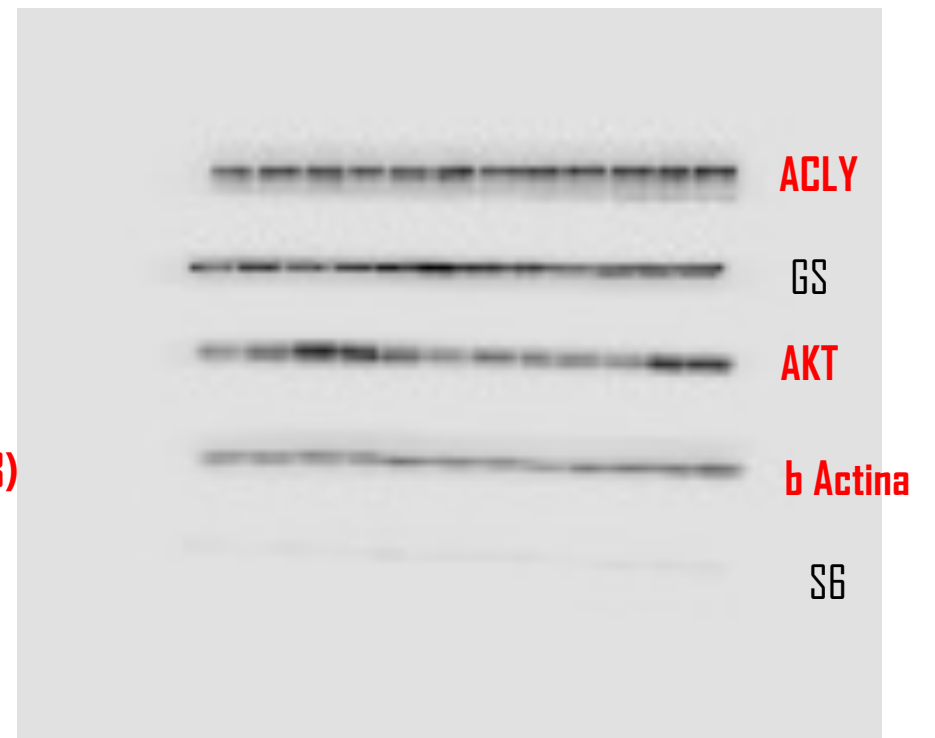
2018

18/06/2018

Gel 1



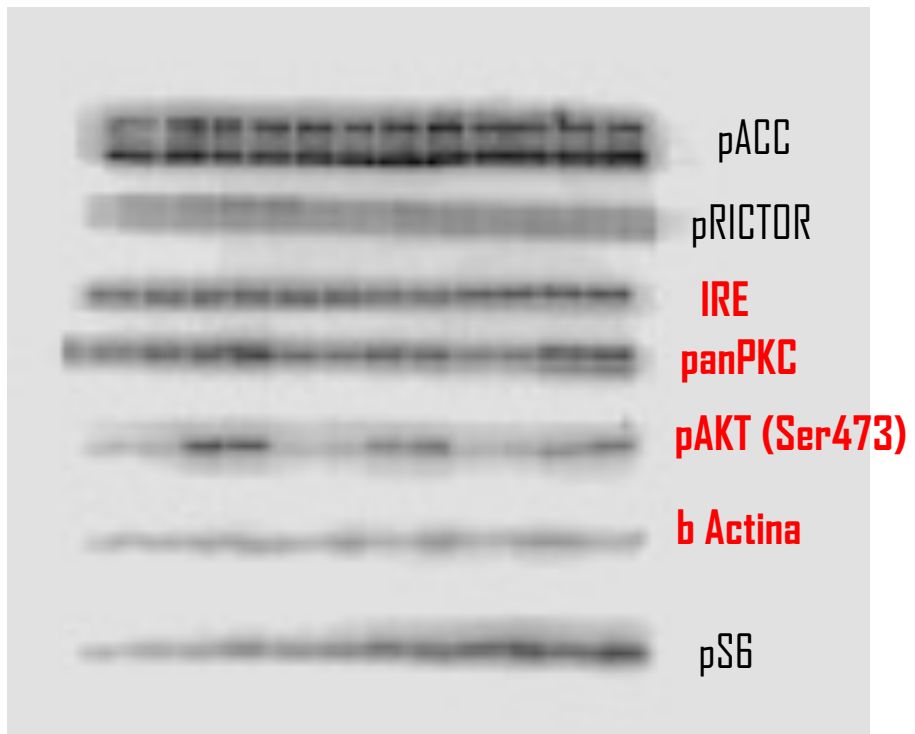
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2018

18/06/2018

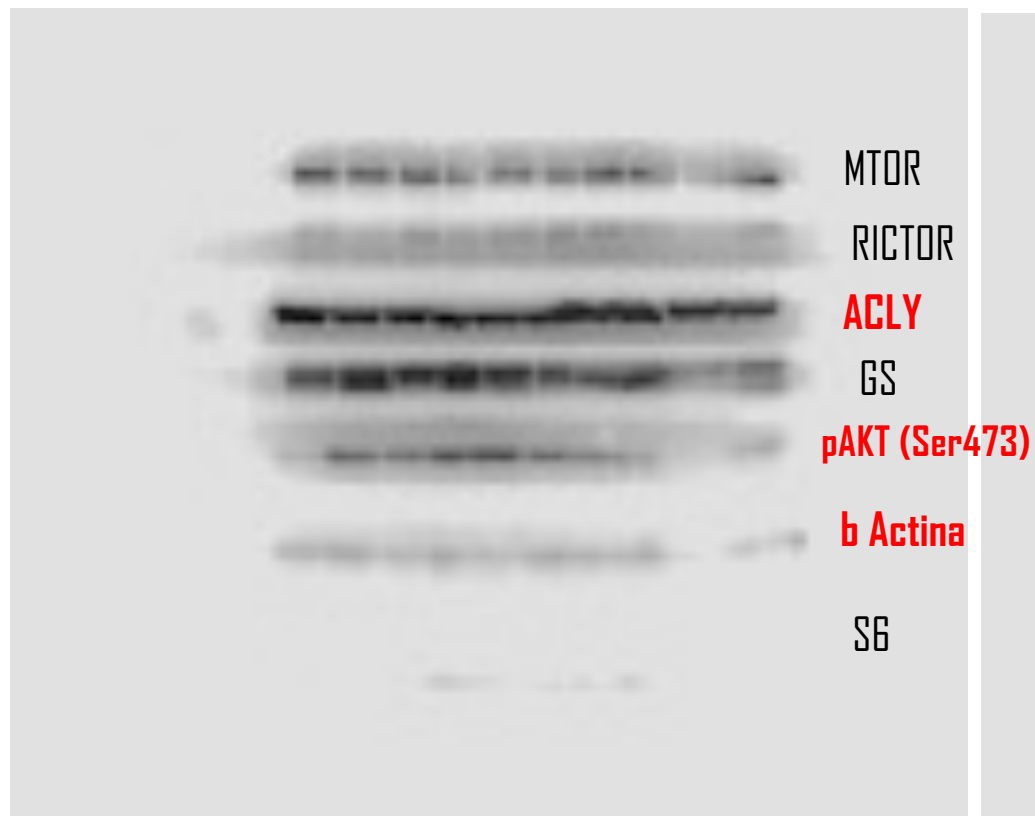
Gel 3



2018

03/09/2018

Gel 1



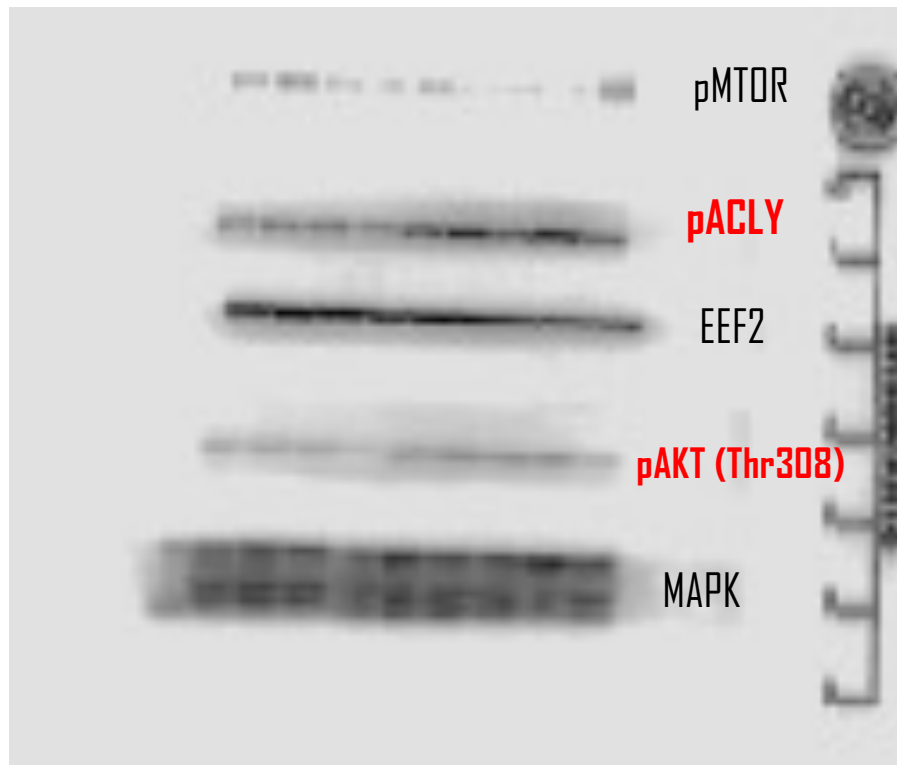
Gel 2



2018

02/10/2018

Gel 1



Gel 2



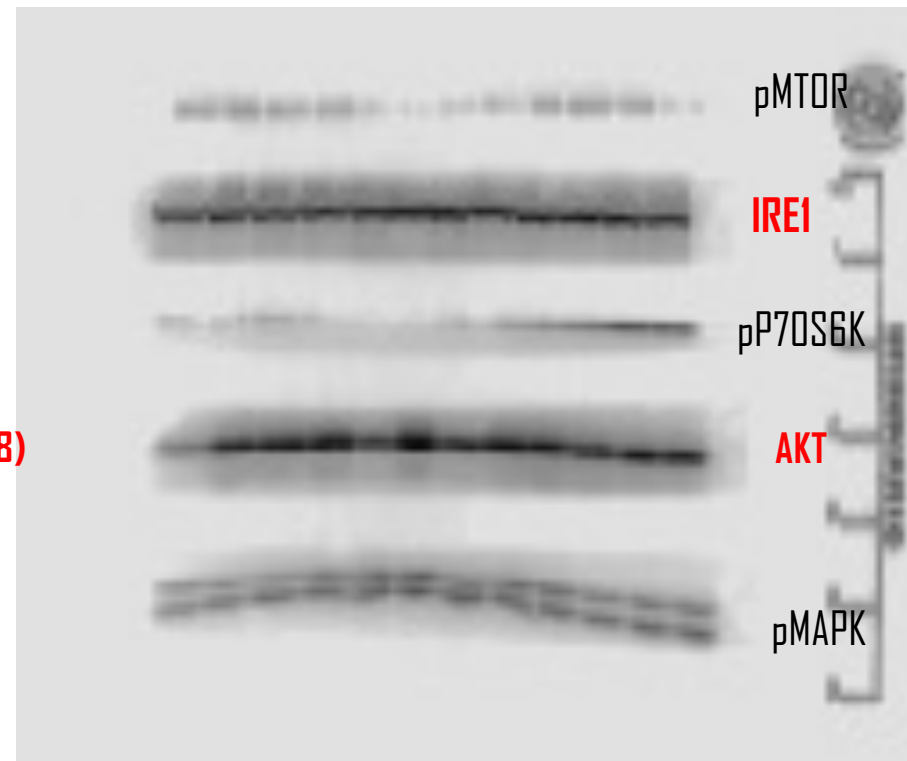
2018

16/10/2018

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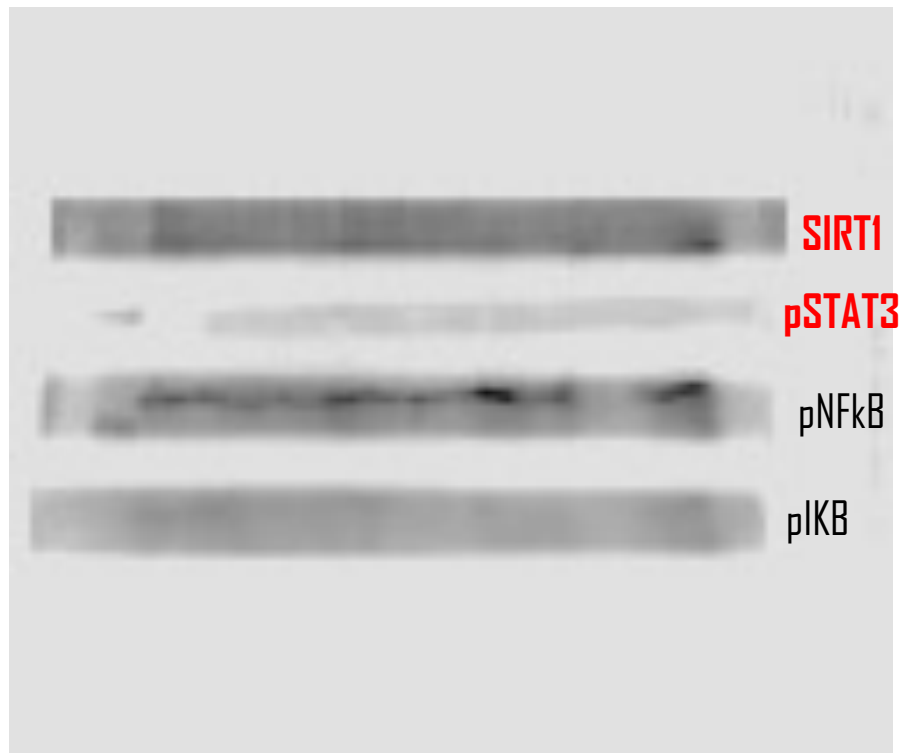
Gel 2



2018

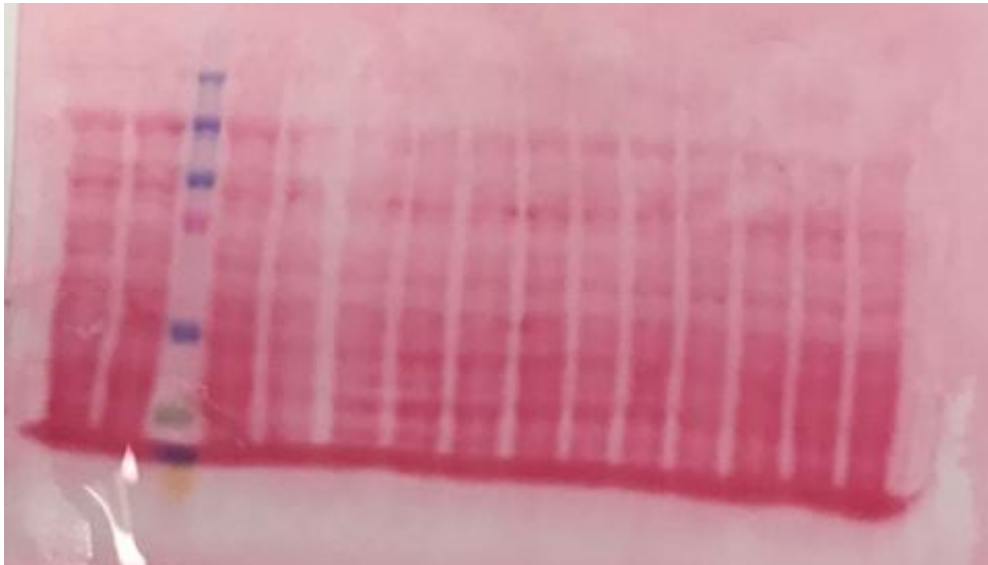
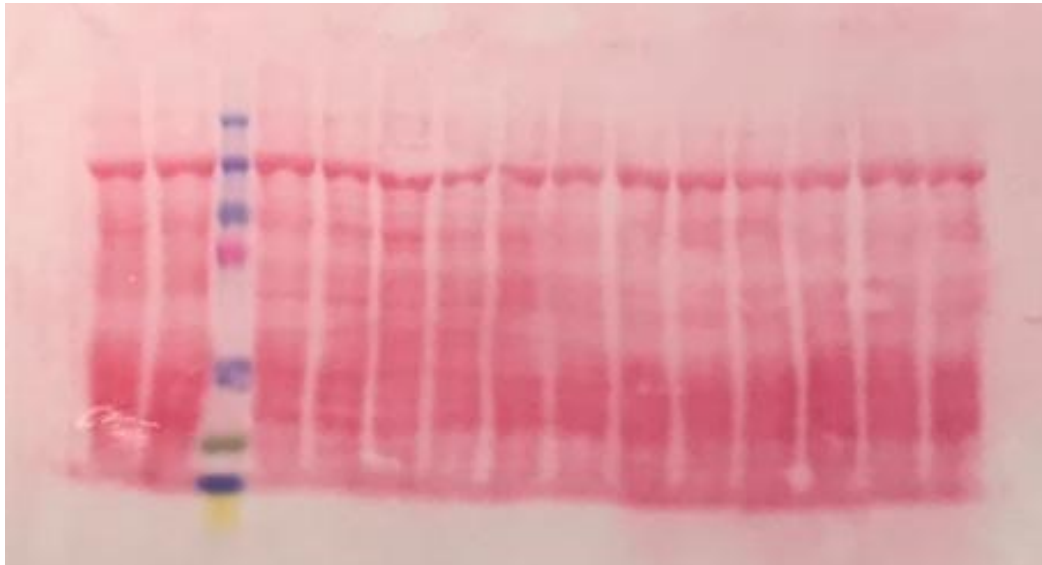
07/11/2018

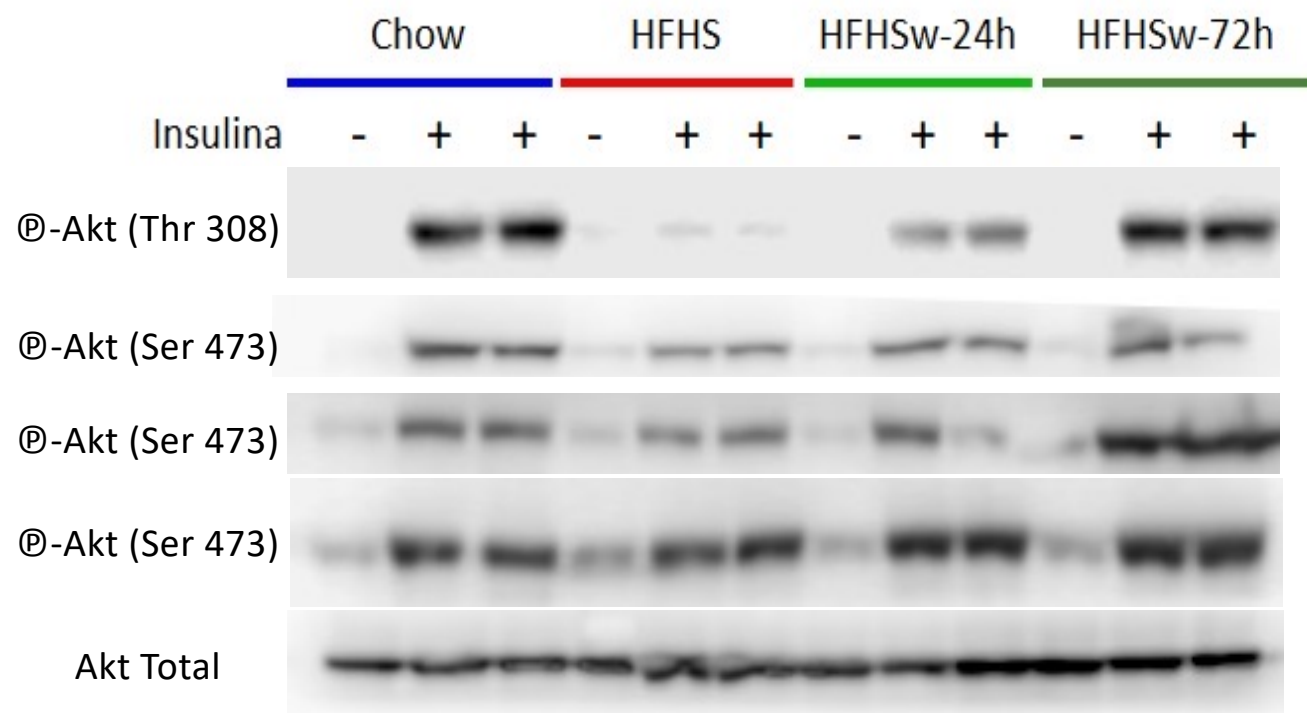
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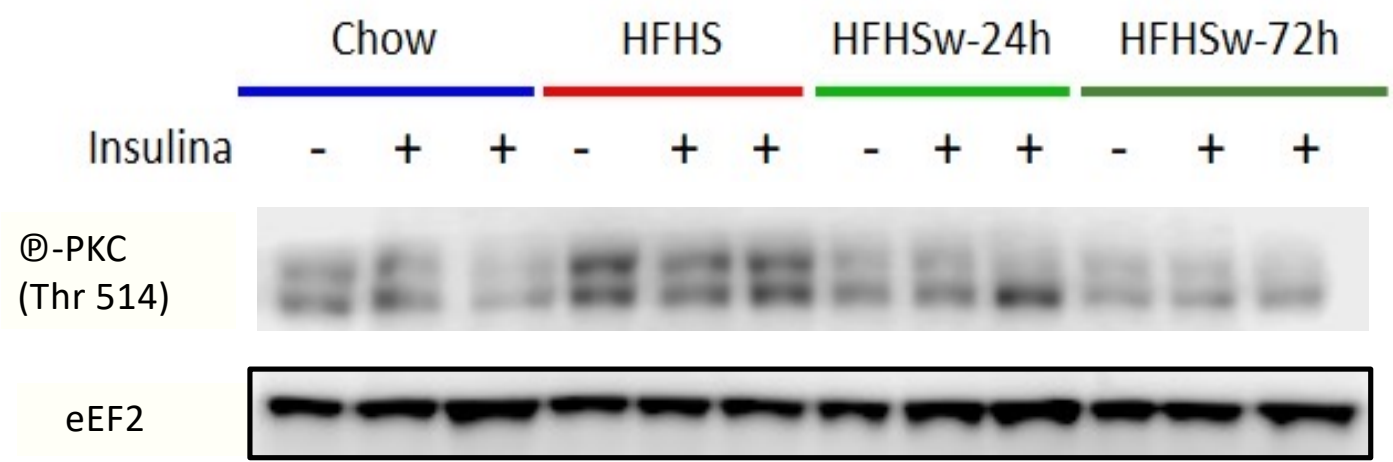


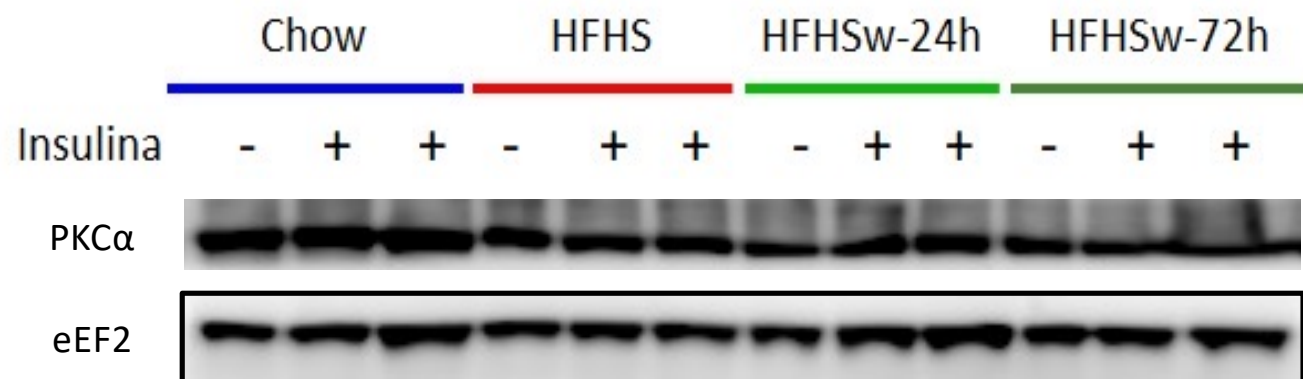
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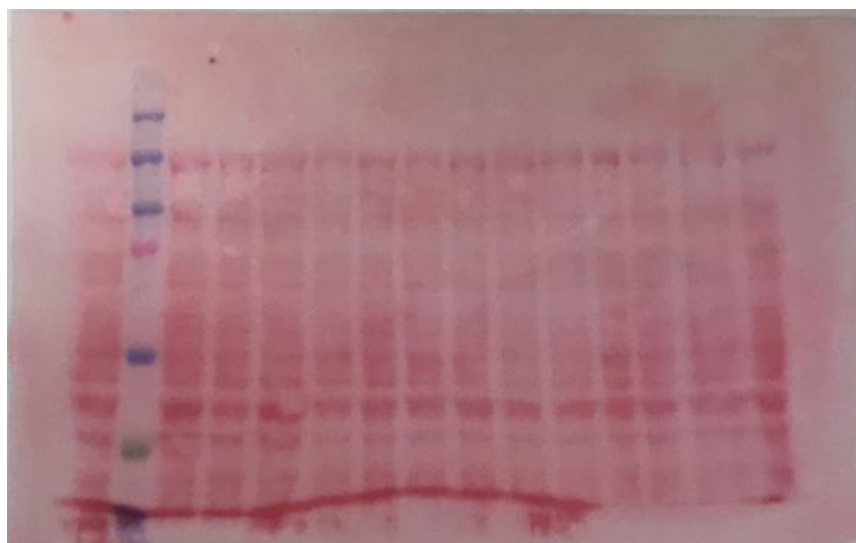


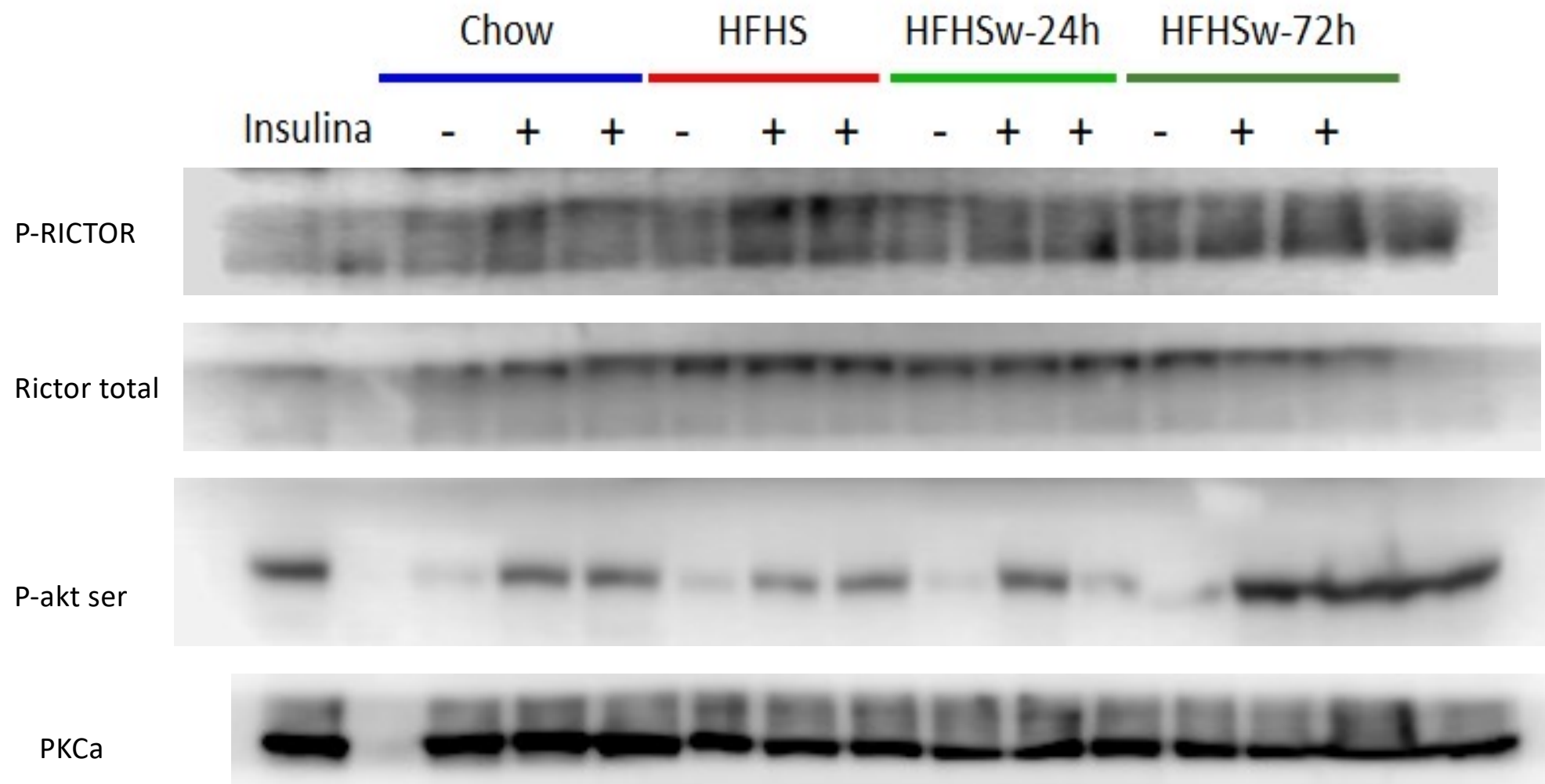


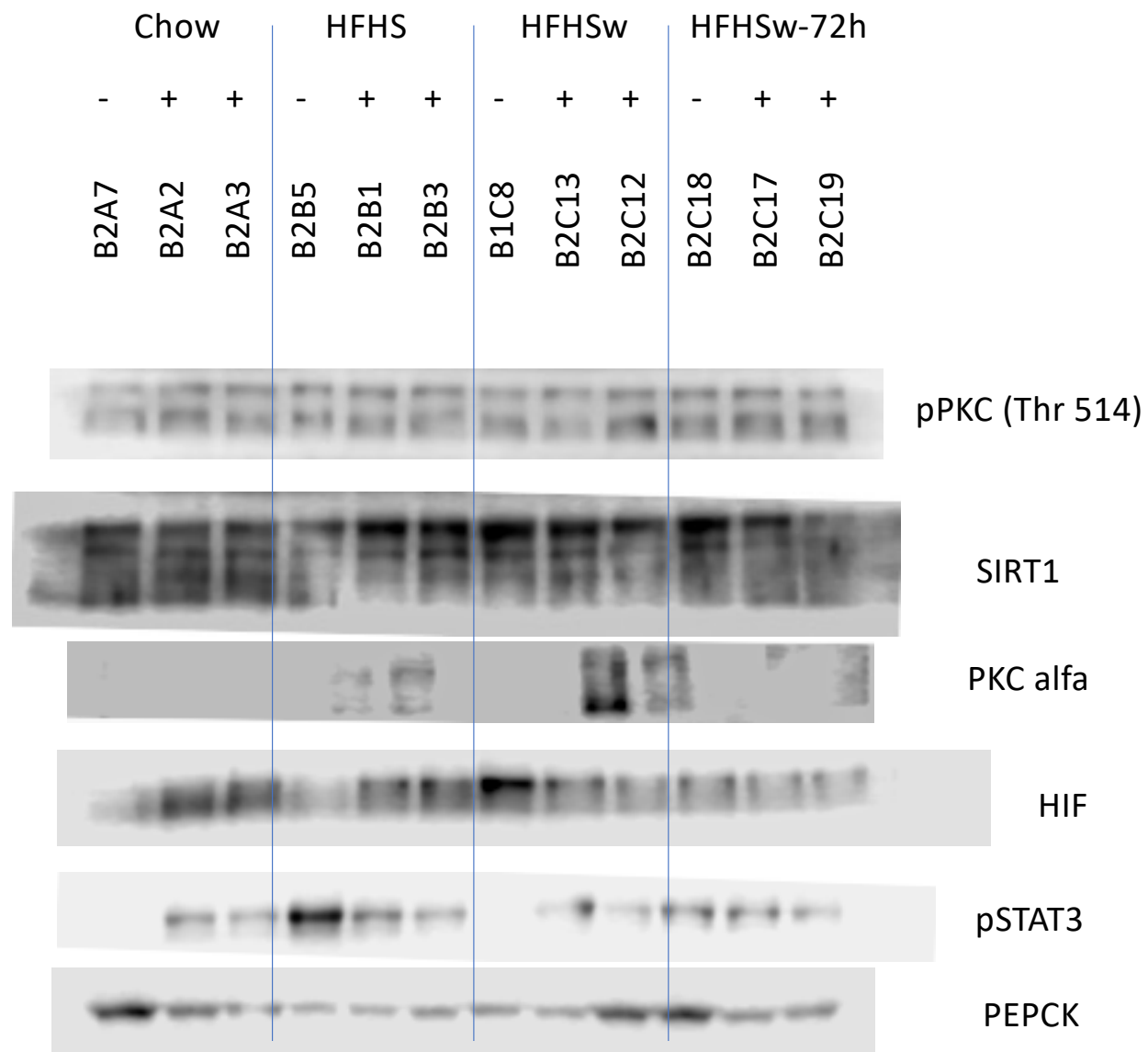


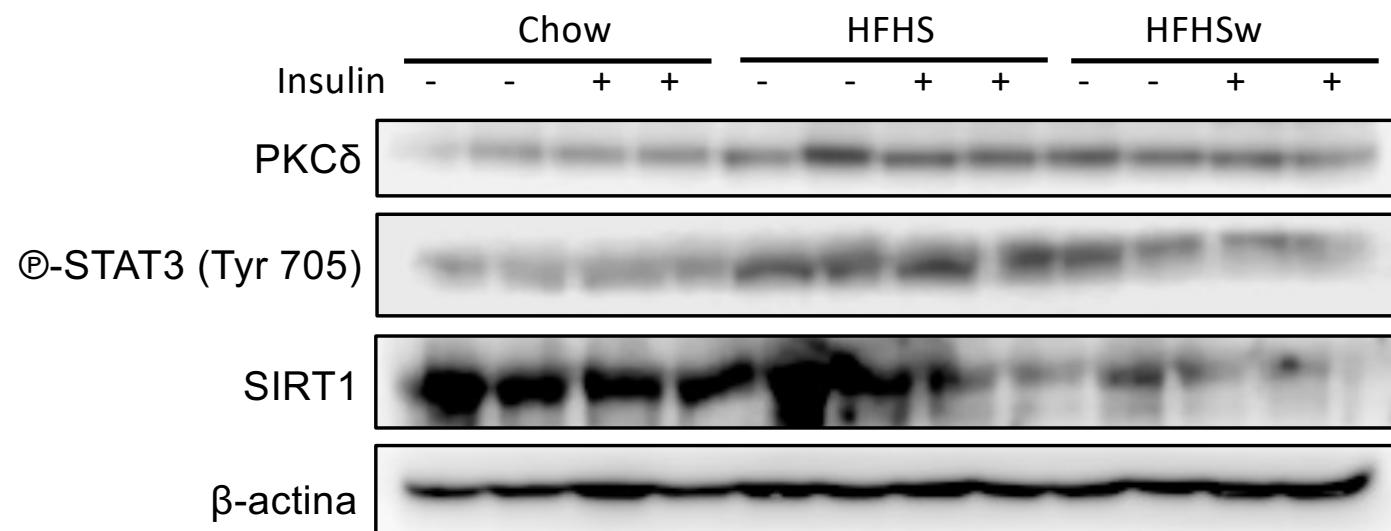


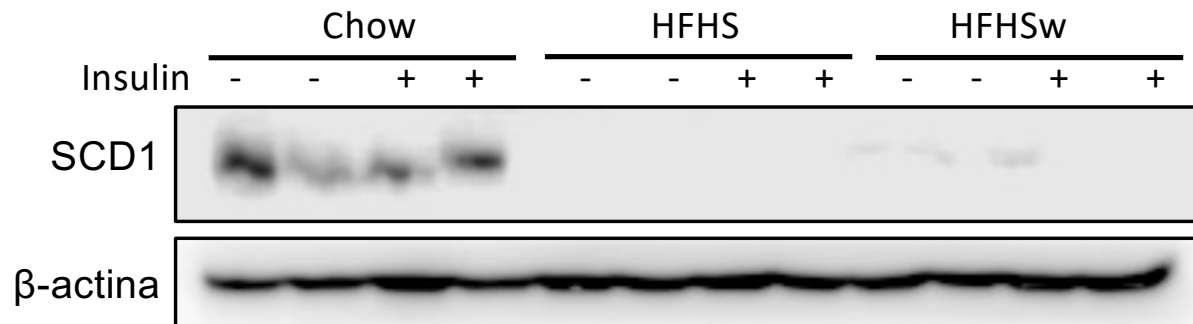
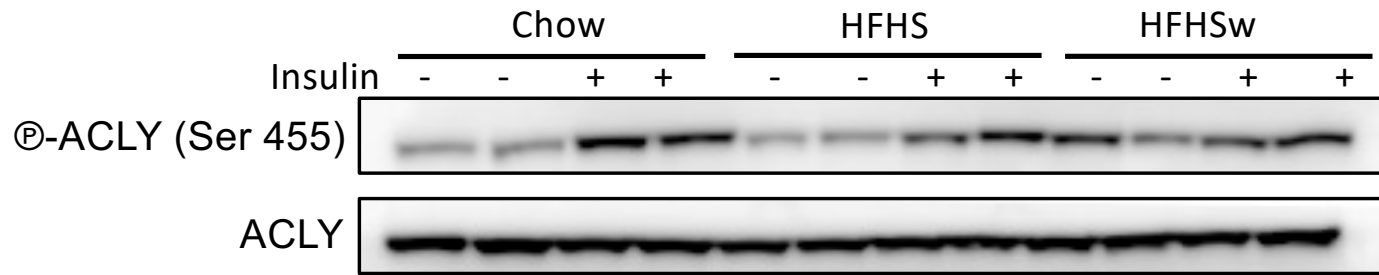


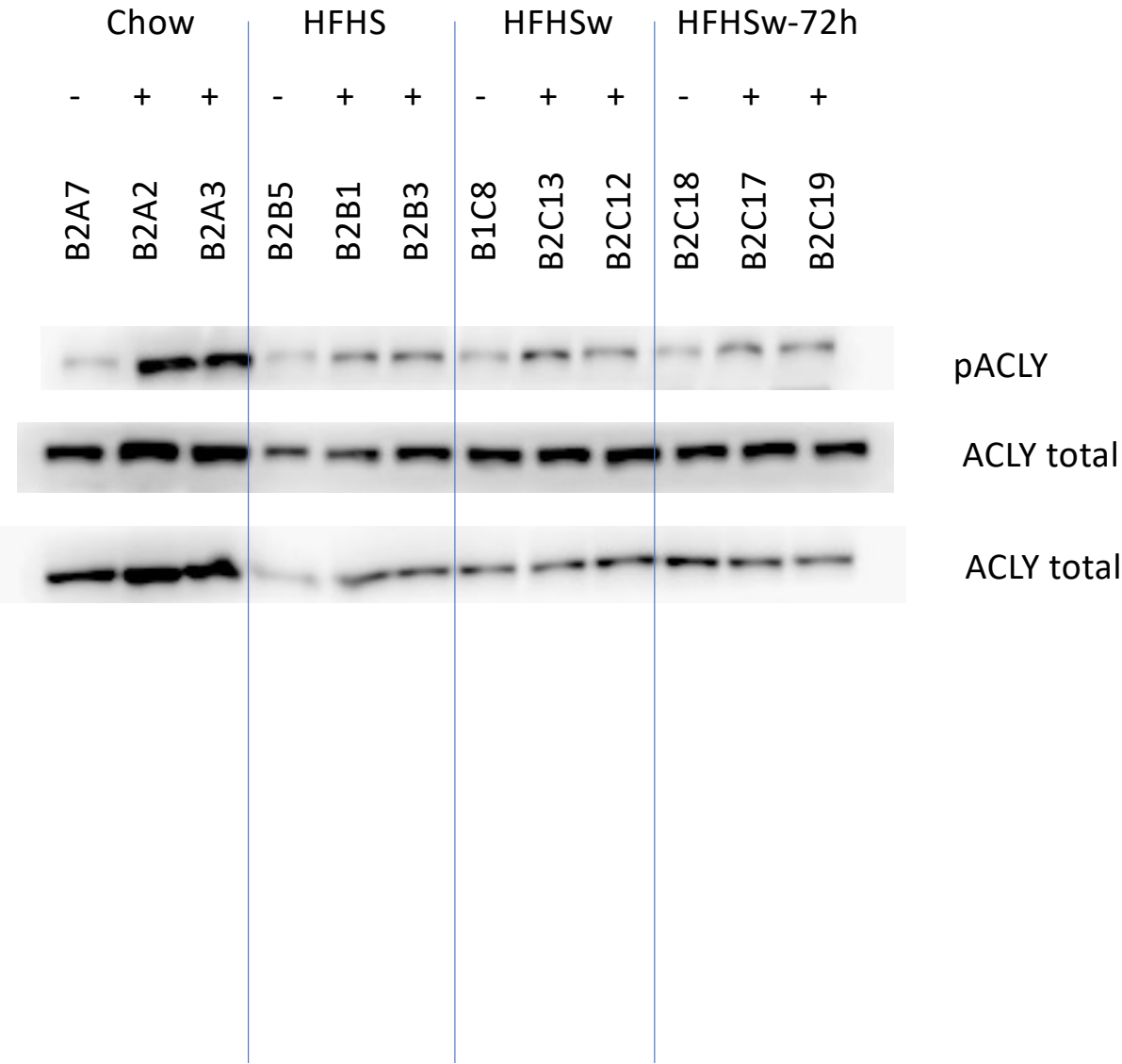


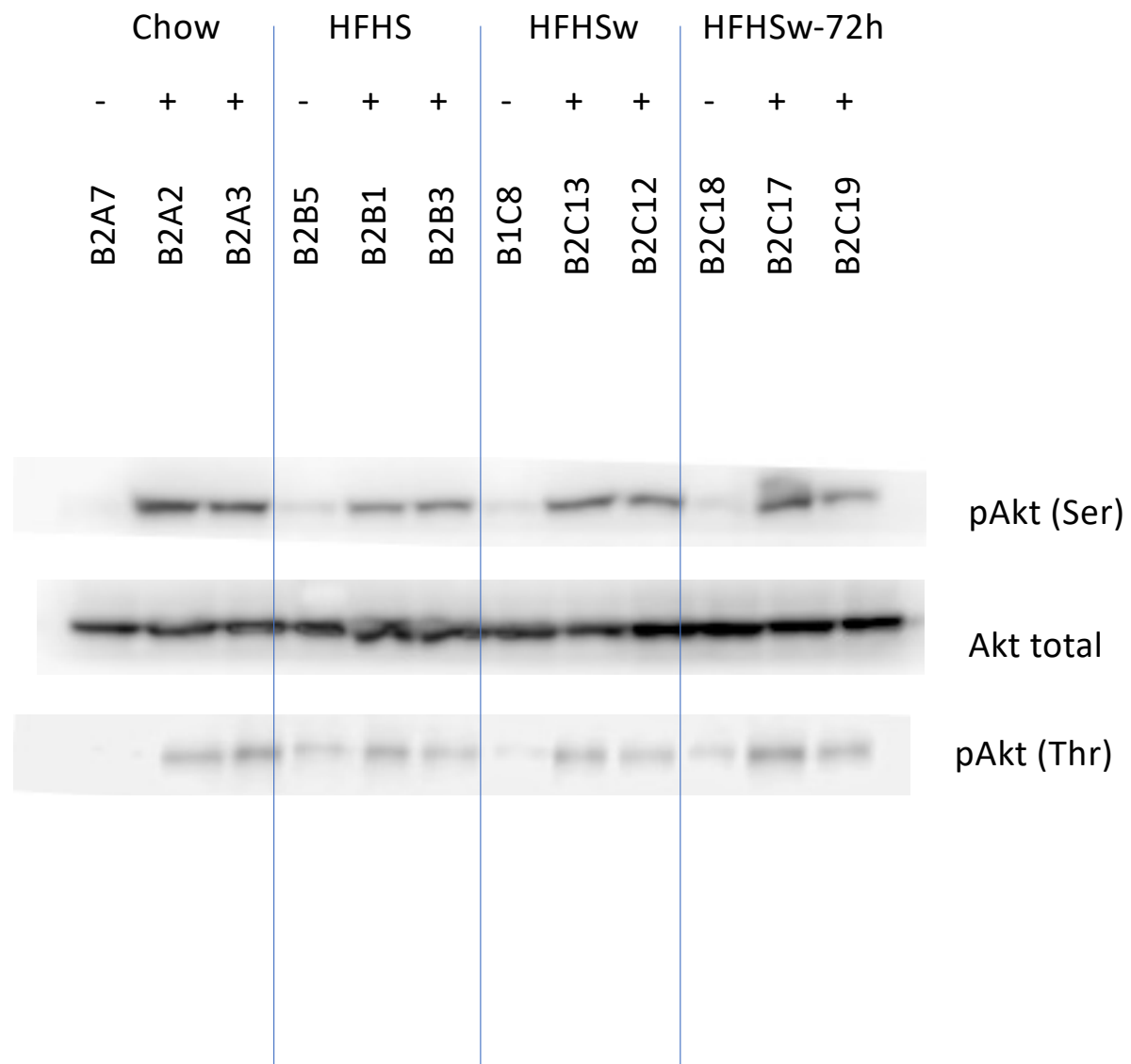


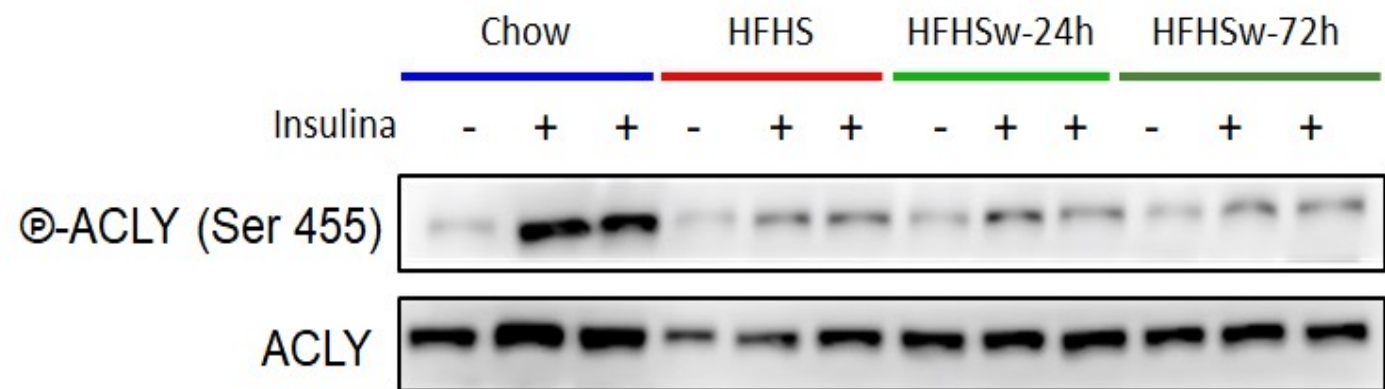
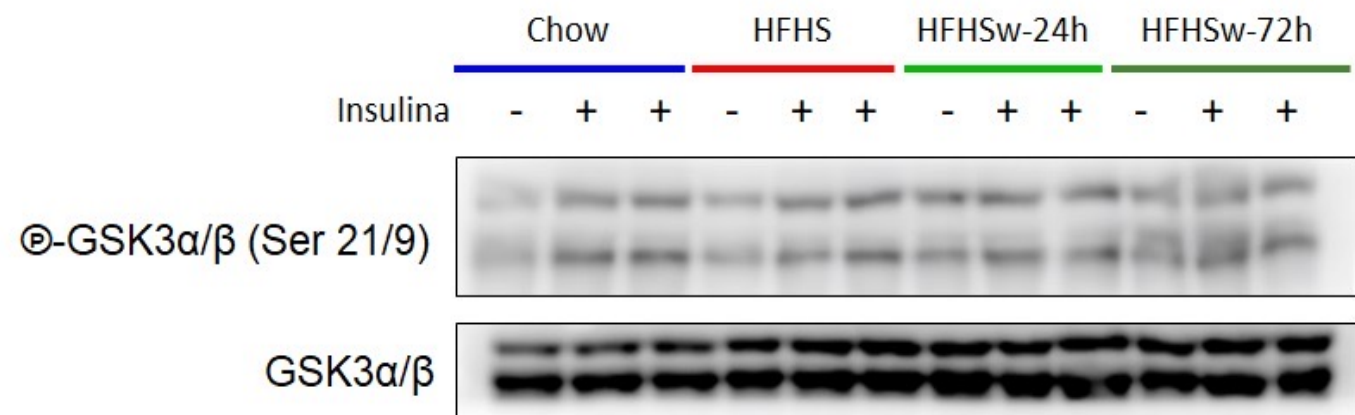


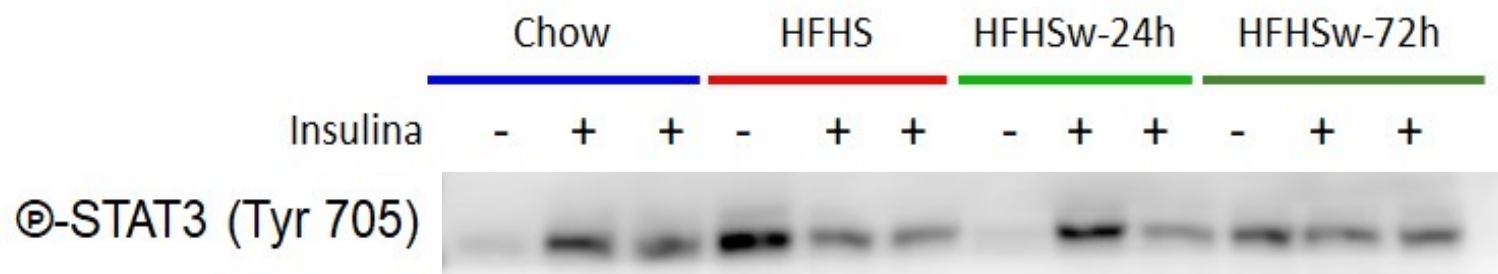
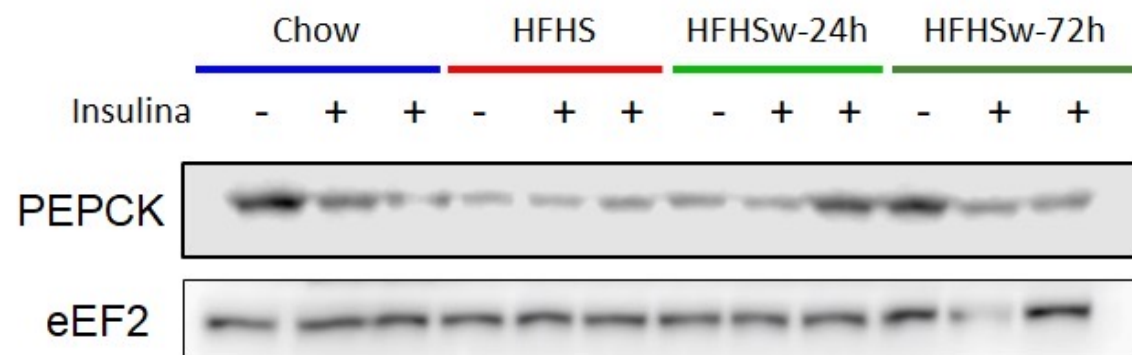


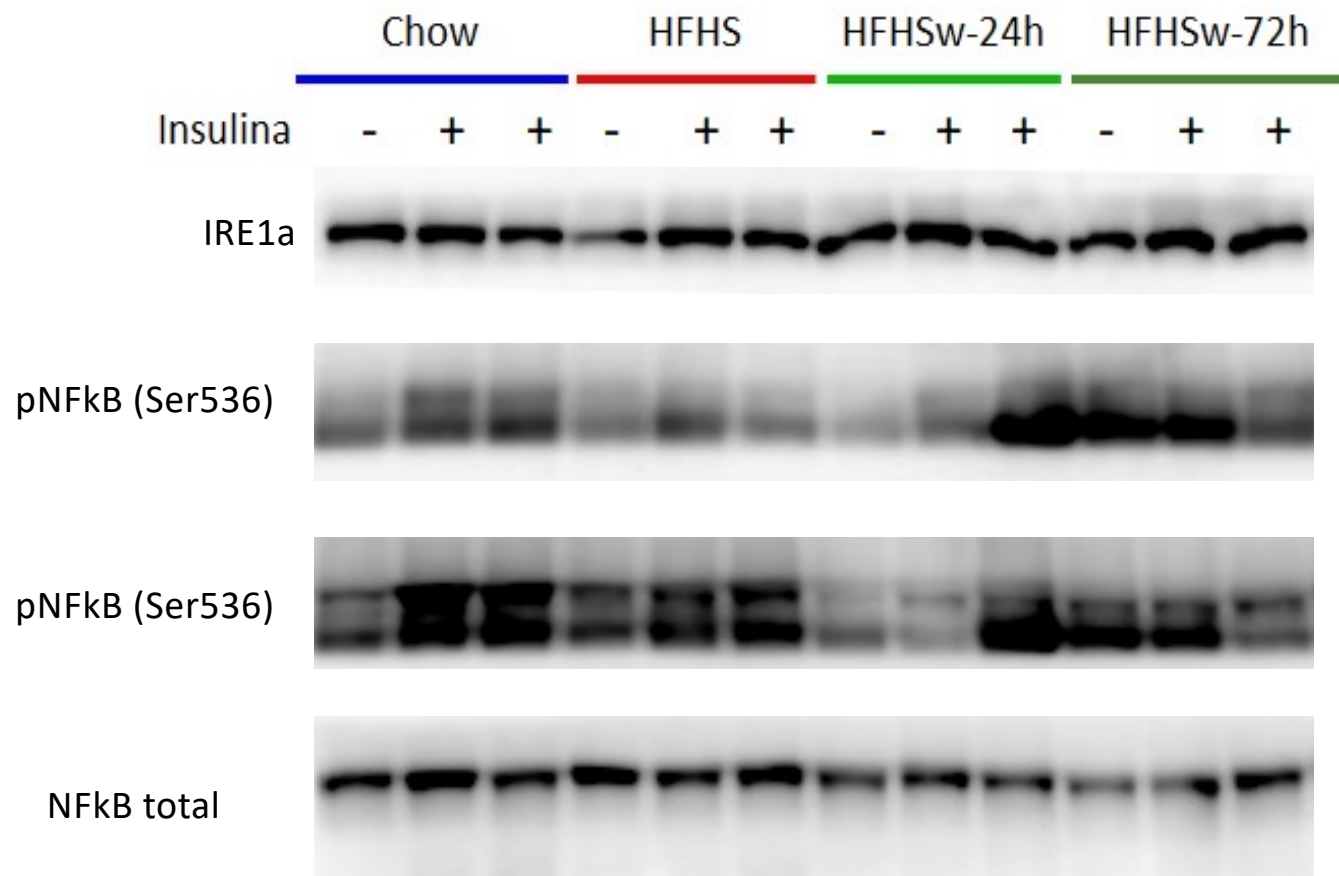


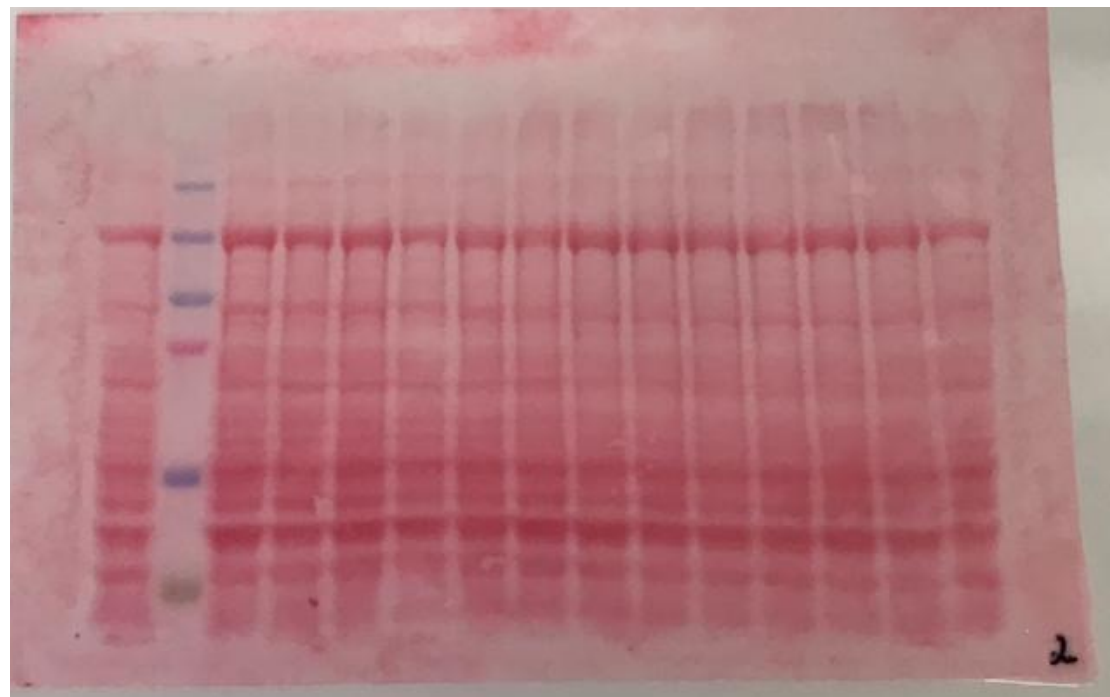
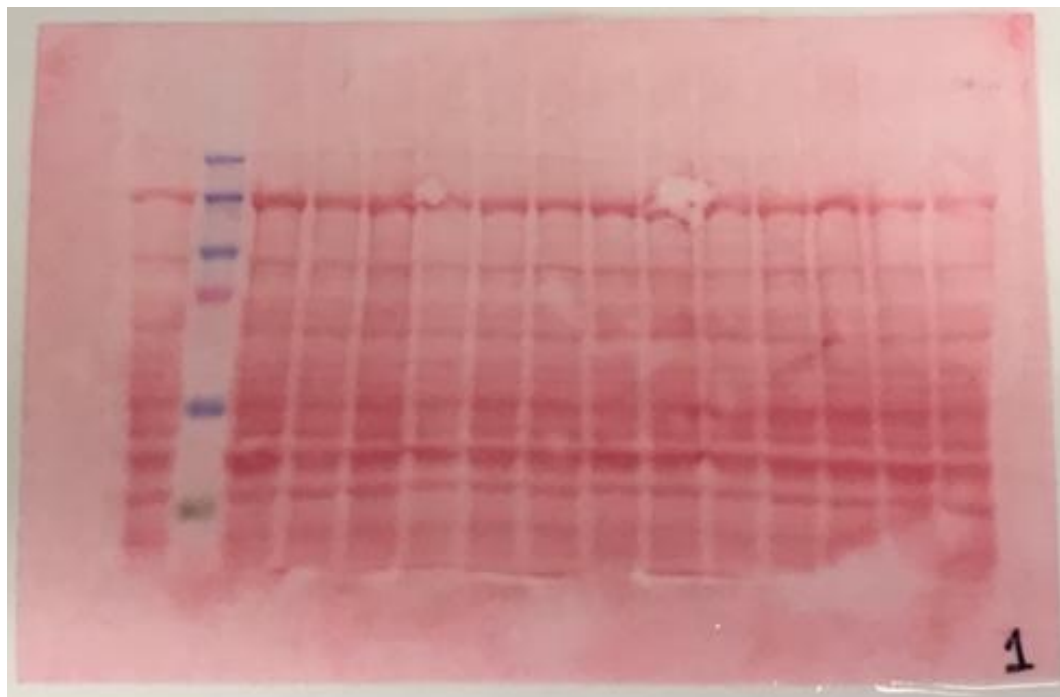






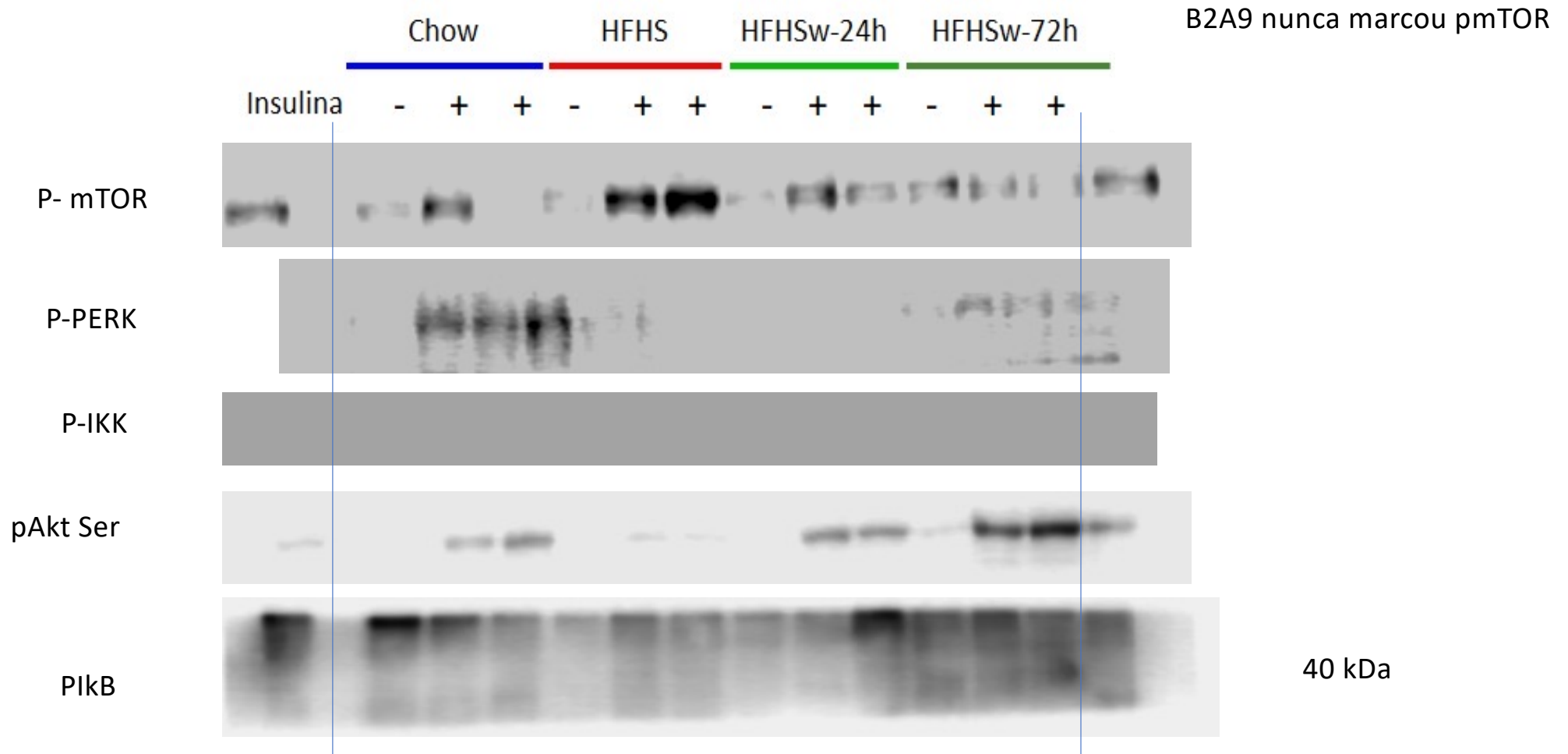






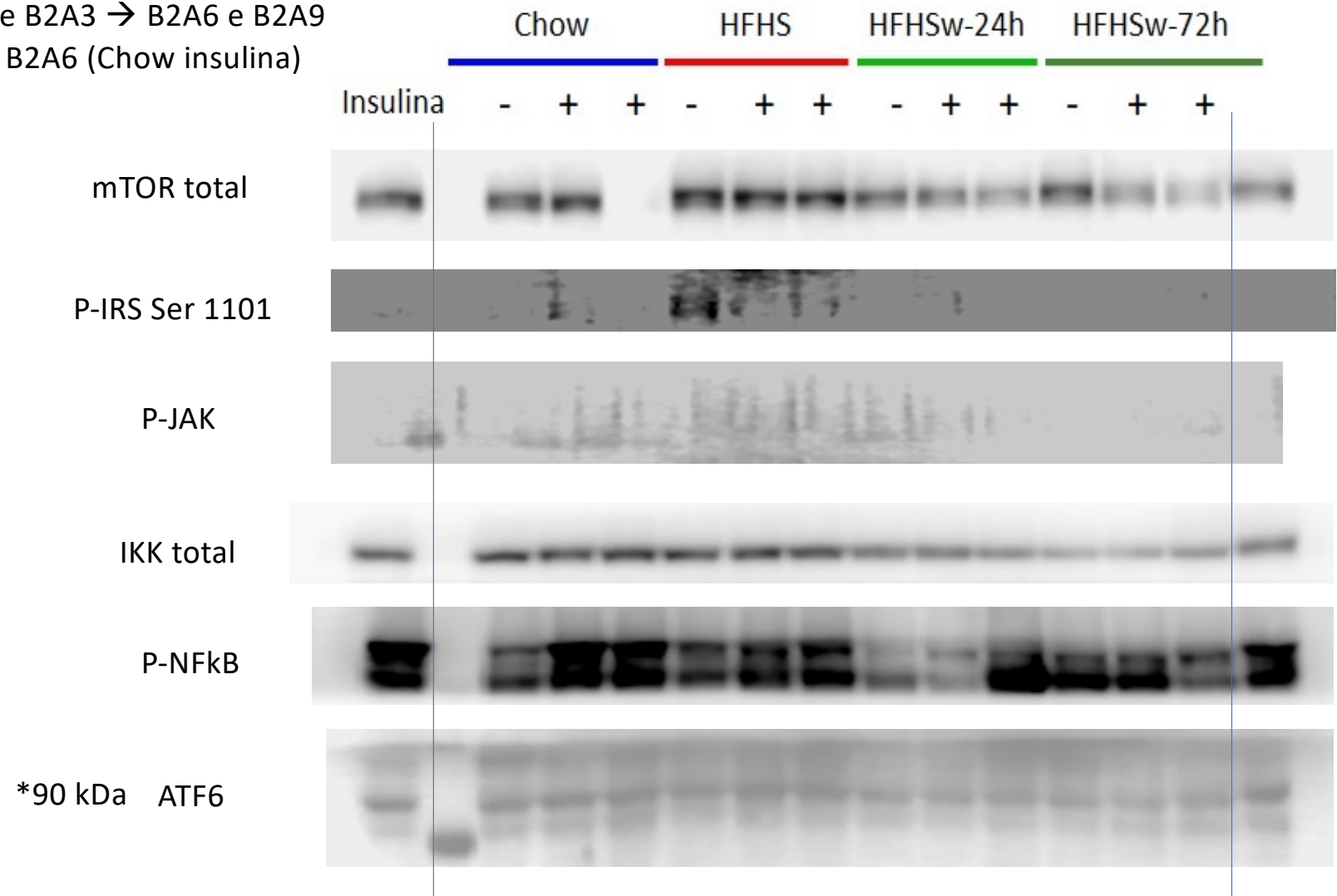
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- Chow insulina B2A2 e B2A3 → B2A6 e B2A9
- Amostras das pontas: B2A6 (Chow insulina)

Gel 1

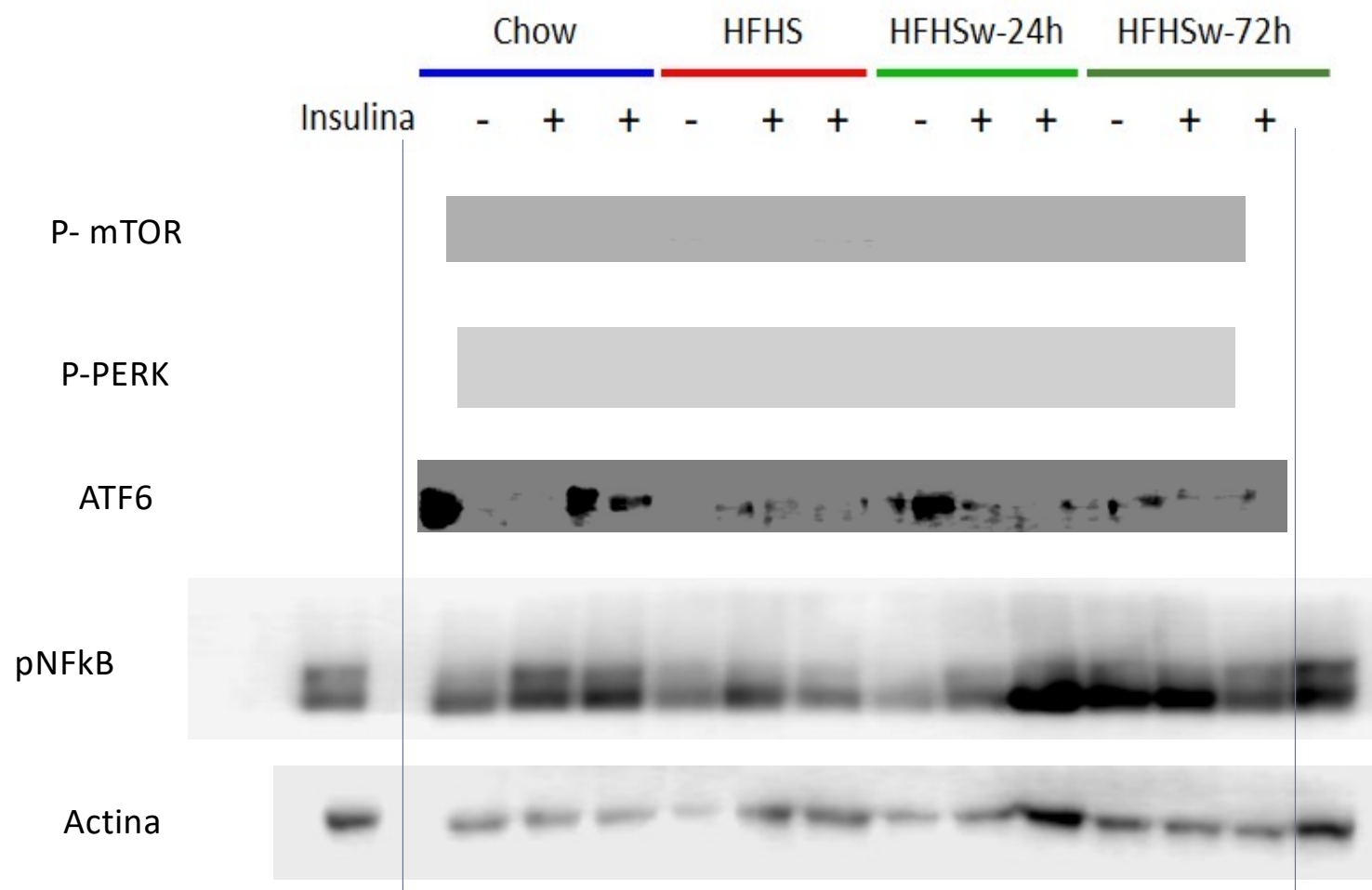


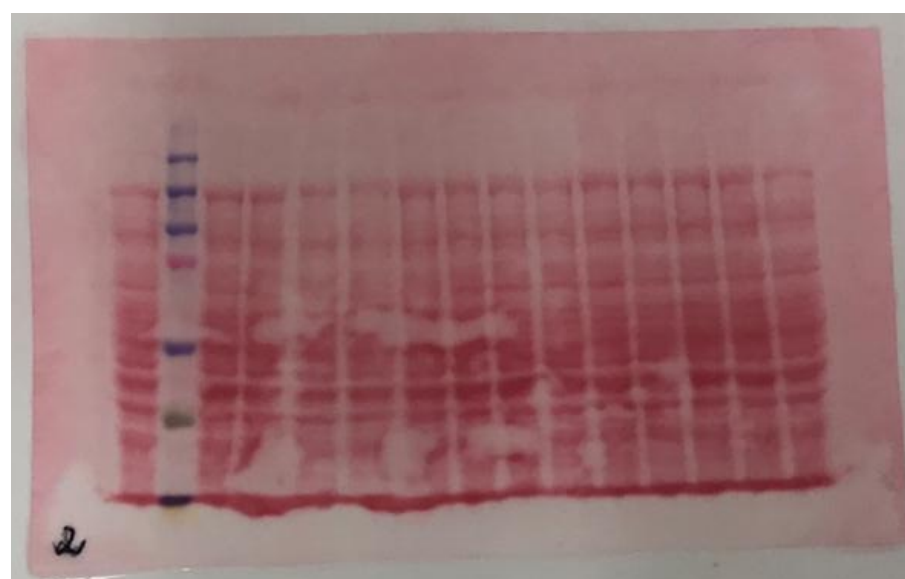
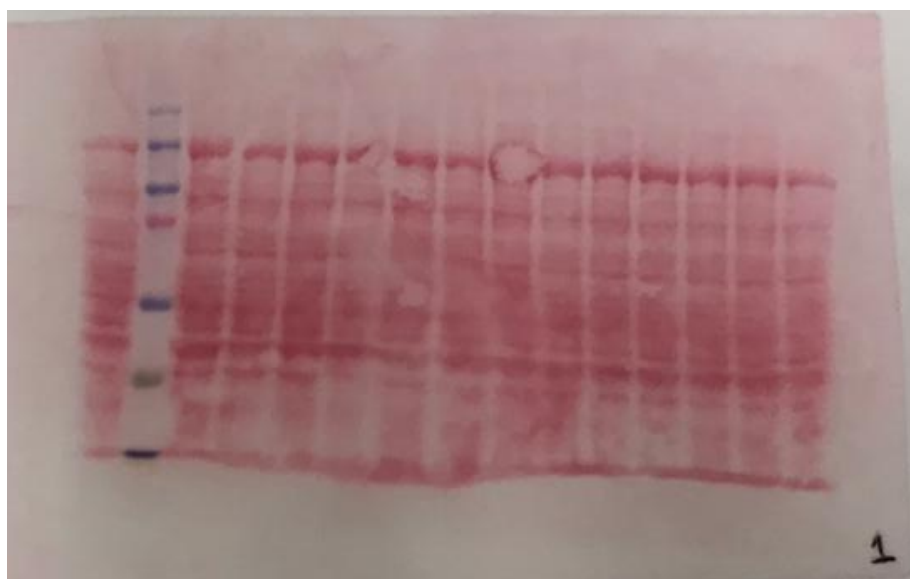
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- Amostras das pontas: B2A6 (Chow insulina)

Gel 2



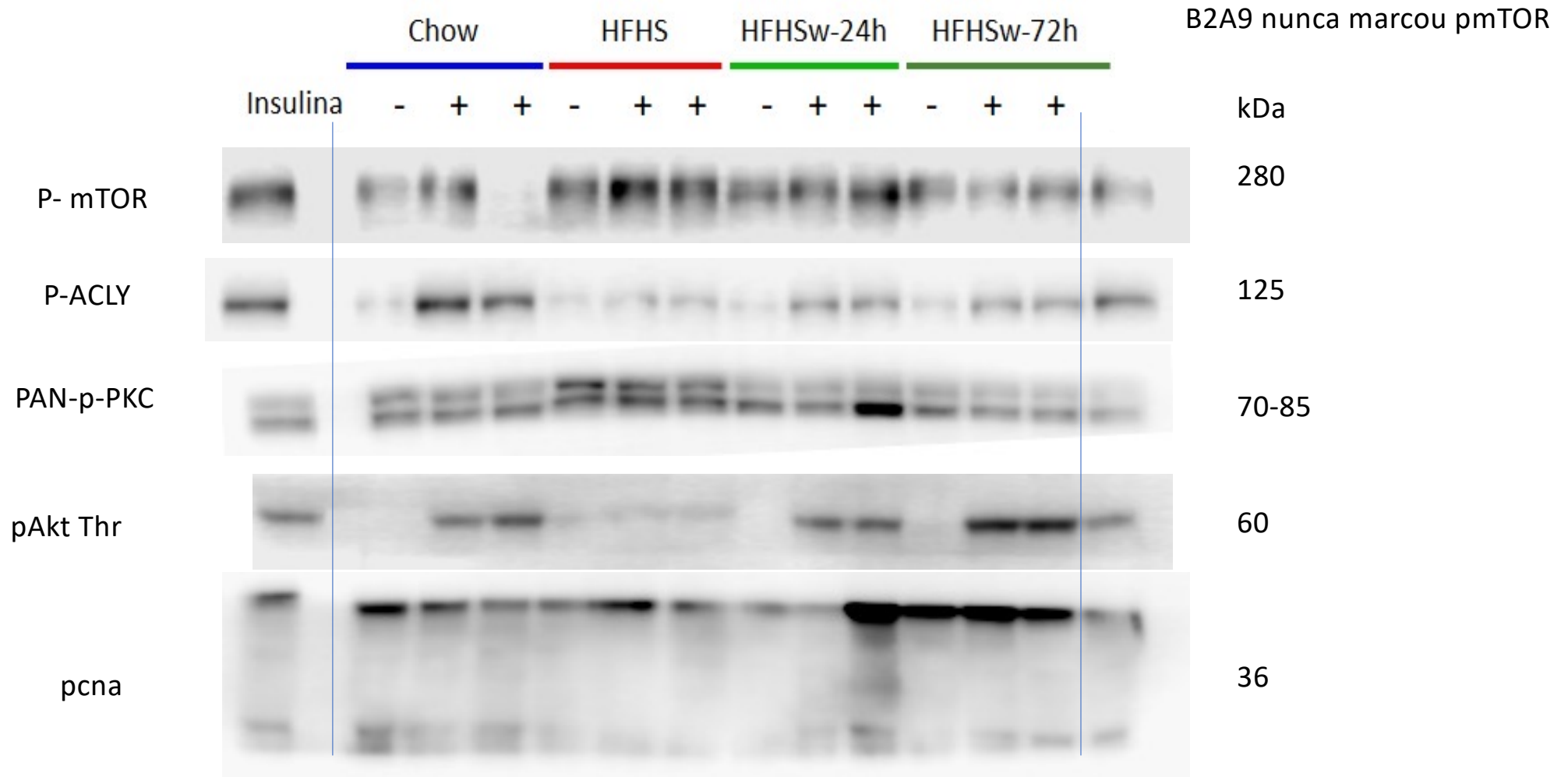
Gel 1 - Stripping

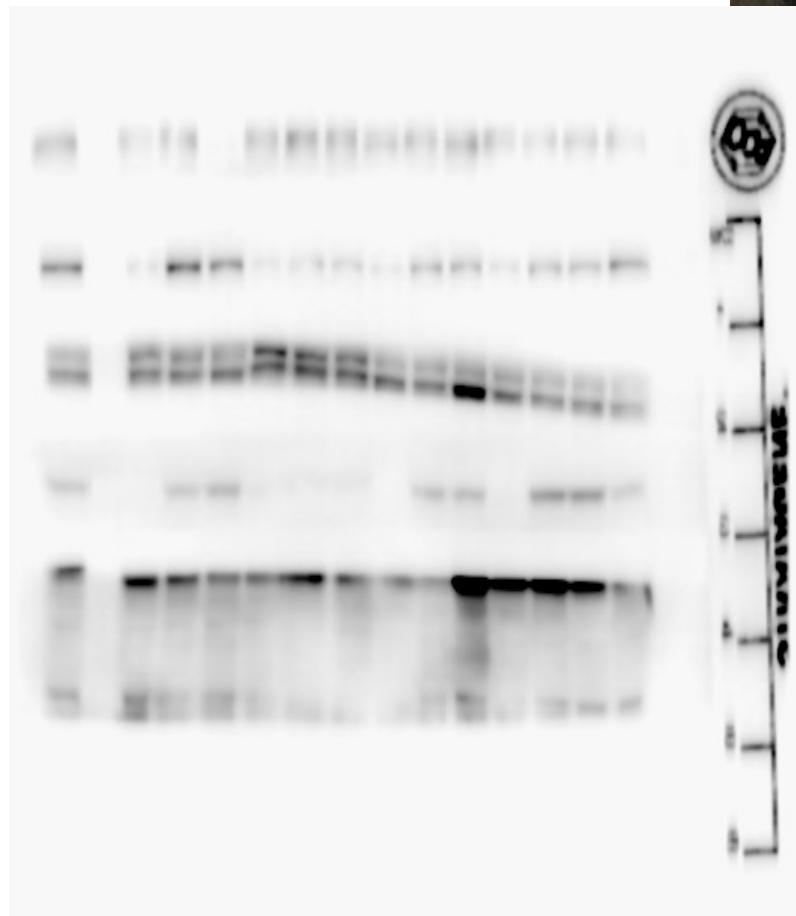


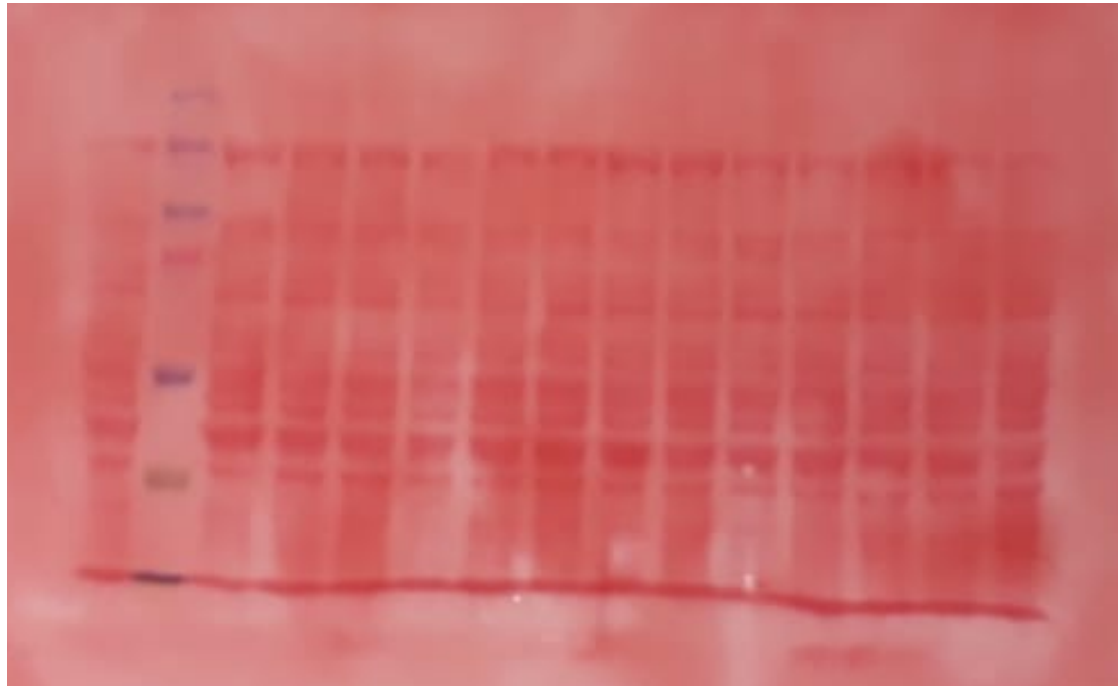


- Amostra Chow salina B2A7 → B1A14
- Chow insulina B2A2 e B2A3 → B2A6 e B2A9
- Amostras das pontas: B2A6 (Chow insulina)

Gel 1







- Amostra Chow salina B2A7 → B1A14
- Amostras das pontas: B2A2 e B2A3 (Chow insulina)

