

## Supplementary Materials

Article

# Antiviral Toll-like Receptor Signaling in Non-Parenchymal Liver Cells Is Restricted to TLR3

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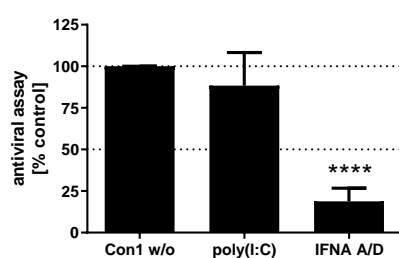
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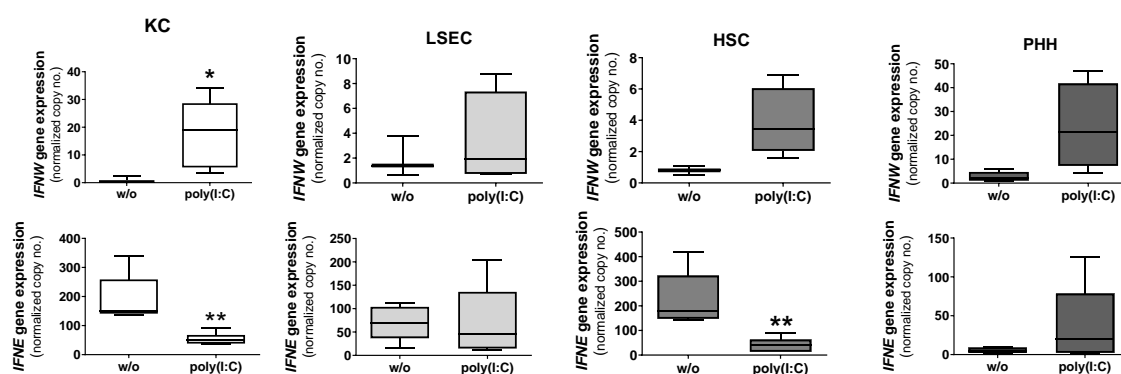
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## Supplementary Figures



**Figure S1. HCV replicon system con1 does not respond to poly(I:C).** Subgenomic HCV replicon system con1 was treated with 50µg/ml poly(I:C) or 10IU/ml recombinant IFNA A/D. RNA was extracted after 72h and HCV replication was determined by qRT-PCR.



**Figure S2. NPC show low expression of IFNW and IFNE.** Gene expression of *IFNW* and *IFNE* was determined in NPC (n=3) treated with poly(I:C) for 6h. Data represent copy numbers as mean ± sd normalized to 100,000 copies of reference gene ACTB. Abbreviations: HSC, hepatic stellate cells; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cells; nd, not detectable; sd, standard deviation; TLR, toll like receptor; w/o (without treatment)

## Supplementary Tables

**Table S1. Patients' characteristics**

KC	Control group (n=15) mean $\pm$ sem	HCV group (n=10) mean $\pm$ sem
transplantation	3	9
resection	12	1
male	8	7
female	7	3
age	54.9 $\pm$ 4.2	55.6 $\pm$ 2.1
GOT [IU/ml]	214.0 $\pm$ 57.7	241.4 $\pm$ 119.6
GPT [IU/ml]	153.4 $\pm$ 37.6	169.9 $\pm$ 77.0
fibrosis stage	1.5 $\pm$ 0.4	3.9 $\pm$ 0.1
LSEC	Control group (n=15) mean $\pm$ sem	HCV group (n=10) mean $\pm$ sem
transplantation	2	10
resection	13	0
male	9	7
female	6	3
age	56.7 $\pm$ 4.6	55.0 $\pm$ 1.1
GOT [IU/ml]	233.9 $\pm$ 64.1	216.1 $\pm$ 115.3
GPT [IU/ml]	141.0 $\pm$ 40.6	163.8 $\pm$ 77.5
fibrosis stage	1.7 $\pm$ 0.4	3.7 $\pm$ 0.3
HSC	Control group (n=15) mean $\pm$ sem	HCV group (n=10) mean $\pm$ sem
transplantation	2	10
resection	13	0
male	5	9
female	10	1
age	58.5 $\pm$ 5.2	53.1 $\pm$ 1.6
GOT [IU/ml]	277.3 $\pm$ 88.6	222.3 $\pm$ 114.1
GPT [IU/ml]	187.1 $\pm$ 61.4	164.7 $\pm$ 77.4
fibrosis stage	2.2 $\pm$ 0.5	3.7 $\pm$ 0.3

Data represent mean values $\pm$ sem. Abbreviations: GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; HCV, hepatitis c virus; HSC, hepatic stellate cells; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cells; sem, standard error of mean.

**Table S2. Primer sequences**

Gene	Forward primer sequence	Reverse primer sequence	Species
<i>ACTB</i>	5'-TCCCTGGAGAAGAGCTACGA-3'	5'-AGCAATGTGTTGGCGTACAG-3'	Hs
<i>HCV</i>	5'-GCGGGTTGATCCAAGAAAGG-3'	5'-ATCACTCCCCTGTGAGGAACT-3'	Hs
<i>MX1</i>	5'-AGCCACTGGACTGACGACTT-3'	5'-GAGGGCTGAAAATCCCTTTC-3'	Hs
<i>Ifi1</i>	5'-CTGAAATGCCAAGTAGCAAGG-3'	5'-CCAAAGGCACAGACATAAGGA-3'	Mm
<i>Gapdh</i>	5'-AAATTCAACGGCACAGTCAA-3'	5'-TCTCCATGGTGGTGAAGACA-3'	Mm

Table S3. Primary and secondary antibodies

Protein	Abbreviation	Company
Interferon regulatory factor 3	IRF3	Abcam
Phospho-interferon regulatory factor 3	P-IRF3	Abcam
Nuclear factor kappa B	NF- $\kappa$ B	Cell Signaling
Phospho-nuclear factor kappa B	P-NF- $\kappa$ B	Cell Signaling
Mitogen-activated protein kinase (MAPK)	MAPK	Cell Signaling
Phospho-mitogen-activated protein kinase	P-MAPK	Cell Signaling
Protein 38	p38	Cell Signaling
Phospho-protein 38	P-p38	Cell Signaling
c-Jun-amino-terminal kinases	JNK	Cell Signaling
Phospho-c-Jun-amino-terminal kinases	P-JNK	Cell Signaling
Serine/threonine-protein kinase	AKT	Cell Signaling
Phospho-serine/threonine-protein kinas	P-AKT	Cell Signaling
Actin beta	ACTB	Sigma
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	GAPDH
Goat anti-rabbit HRP-conjugated	-	Cell Signaling
Goat anti-mouse HRP-conjugated	-	Thermo Scientific

Abbreviations: HRP, horseradish peroxidase

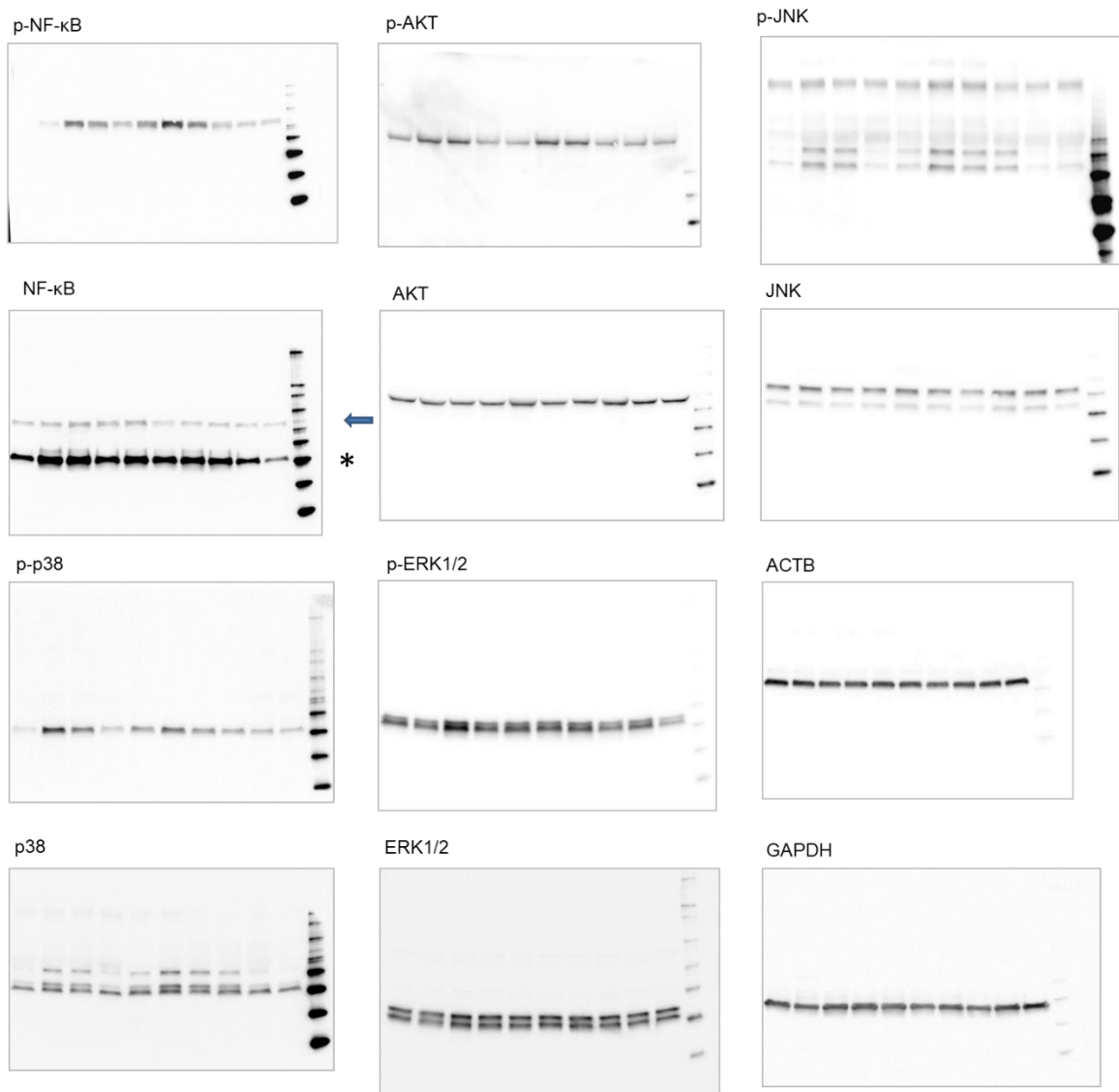
Table S4. TLR-induced gene expression of inflammatory cytokines

KC		IL6		TNF		IL10	
Stimulation	mean ± sd	p-value	mean ± sd	p-value	mean ± sd	p-value	
w/o	9,862.3 ± 8,243.6		352.7 ± 164.2		148.9 ± 129.4		
Pam3CSK4	35,275.5 ± 19,675.9	<0.001	2,509.5 ± 1,531.8	<0.001	233.4 ± 121.6	0.071	
HKLM	47,645.5 ± 30,411.1	<0.001	4,297.4 ± 3,641.0	<0.001	467.4 ± 268.4	<0.001	
poly(I:C)	27,112.3 ± 16,397.4	0.001	2,523.1 ± 1,394.3	<0.001	151.8 ± 102.2	0.946	
LPS	49,002.0 ± 34,574.1	<0.001	2,833.9 ± 2,204.5	<0.001	346.7 ± 305.2	0.026	
flagellin	87,368.8 ± 44,872.0	<0.001	10,402.4 ± 8,008.9	<0.001	544.9 ± 309.8	<0.001	
FSL-I	39,569.6 ± 23,103.9	<0.001	3,599.6 ± 3,697.9	0.002	270.0 ± 177.0	0.037	
Gdq	56,604.8 ± 38,768.2	<0.001	9,387.8 ± 5,379.6	<0.001	533.9 ± 345.9	<0.001	
ssRN40	24,331.4 ± 12,833.9	0.001	3,358.6 ± 2,831.5	<0.001	233.5 ± 209.5	0.190	
ODN2216	12,280.9 ± 10,063.2	0.474	695.6 ± 290.5	<0.001	178.2 ± 139.0	0.552	
LSEC		IL6		TNF		IL10	
Stimulation	mean ± sd	p-value	mean ± sd	p-value	mean ± sd	p-value	
w/o	3,156.1 ± 2,819.5		244.0 ± 180.5		40.3 ± 35.6		
Pam3CSK4	4,428.9 ± 2,866.8	0.225	512.4 ± 388.8	0.020	52.0 ± 46.9	0.446	
HKLM	4,160.7 ± 3,022.5	0.350	522.4 ± 399.7	0.018	46.6 ± 43.4	0.667	
poly(I:C)	13,250.3 ± 11,861.0	0.003	720.9 ± 496.9	0.001	74.1 ± 55.0	0.065	
LPS	5,853.4 ± 3,562.4	0.025	710.6 ± 602.2	0.007	58.3 ± 46.1	0.240	
flagellin	14,118.1 ± 19,502.0	0.039	896.8 ± 576.7	<0.001	94.1 ± 72.4	0.013	
FSL-I	7,576.4 ± 3,537.6	<0.001	791.0 ± 500.8	<0.001	64.7 ± 48.4	0.125	
Gdq	4,165.8 ± 2,442.7	0.299	326.6 ± 326.1	0.395	44.0 ± 38.0	0.784	
ssRN40	3,944.8 ± 2,480.3	0.419	493.5 ± 397.4	0.032	34.7 ± 20.1	0.601	
ODN2216	2,527.5 ± 1,969.0	0.482	216.0 ± 154.9	0.650	32.9 ± 24.4	0.521	
HSC		IL6		TNF		IL10	
Stimulation	mean ± sd	p-value	mean ± sd	p-value	mean ± sd	p-value	
w/o	11,396.2 ± 5,376.1		61.3 ± 60.0		12.2 ± 13.6		
Pam3CSK4	38,148.5 ± 18,017.5	<0.001	316.9 ± 324.9	0.005	25.2 ± 33.7	0.175	
HKLM	40,810.2 ± 21,262.3	<0.001	810.0 ± 1,059.6	0.011	43.5 ± 51.9	0.032	
poly(I:C)	54,436.2 ± 23,117.5	<0.001	152.5 ± 156.5	0.046	22.2 ± 22.5	0.198	
LPS	63,691.4 ± 27,297.2	<0.001	476.6 ± 634.8	0.017	41.1 ± 45.7	0.035	
flagellin	132,965.1 ± 110,010.5	<0.001	899.9 ± 819.9	<0.001	57.0 ± 55.8	0.006	
FSL-I	53,066.1 ± 52,041.3	0.004	319.3 ± 300.2	0.003	24.4 ± 22.1	0.089	
Gdq	51,273.5 ± 50,818.6	0.005	489.7 ± 626.3	0.013	24.0 ± 22.9	0.124	
ssRN40	31,228.8 ± 33,671.3	0.032	248.1 ± 338.9	0.044	20.2 ± 16.7	0.199	
ODN2216	15,212.0 ± 11,629.0	0.255	71.5 ± 53.1	0.631	13.8 ± 13.2	0.774	

Gene expression of inflammatory cytokines was determined in untreated NPC (w/o) or in cells treated with TLR ligands for 6h (n=15). Data represent copy numbers as mean ± sd normalized to 100,000 copies of reference gene *ACTB*. Abbreviations: FSL-I; synthetic lipoprotein (TLR2/6 ligand); Gdq, Gardiquimod (TLR7 ligand); HKLM, Heat Killed *Listeria monocytogenes* (TLR2 ligand); HSC, hepatic stellate cells; KC, Kupffer cells; LPS, lipopolysaccharide (TLR4 ligand); LSEC, liver sinusoidal endothelial cells; ODN2216, synthetic single-stranded DNA (TLR9 ligand); Pam3CSK4, synthetic triacylated lipopeptide (TLR1/2 ligand); poly(I:C), polyinosinic-polycytidylic acid (TLR3 ligand); sd, standard deviation; ssRNA40, single-stranded RNA oligonucleotide (TLR8 ligand); TLR, toll like receptor; w/o (without treatment)

## Original western blots - Figure 1

KC

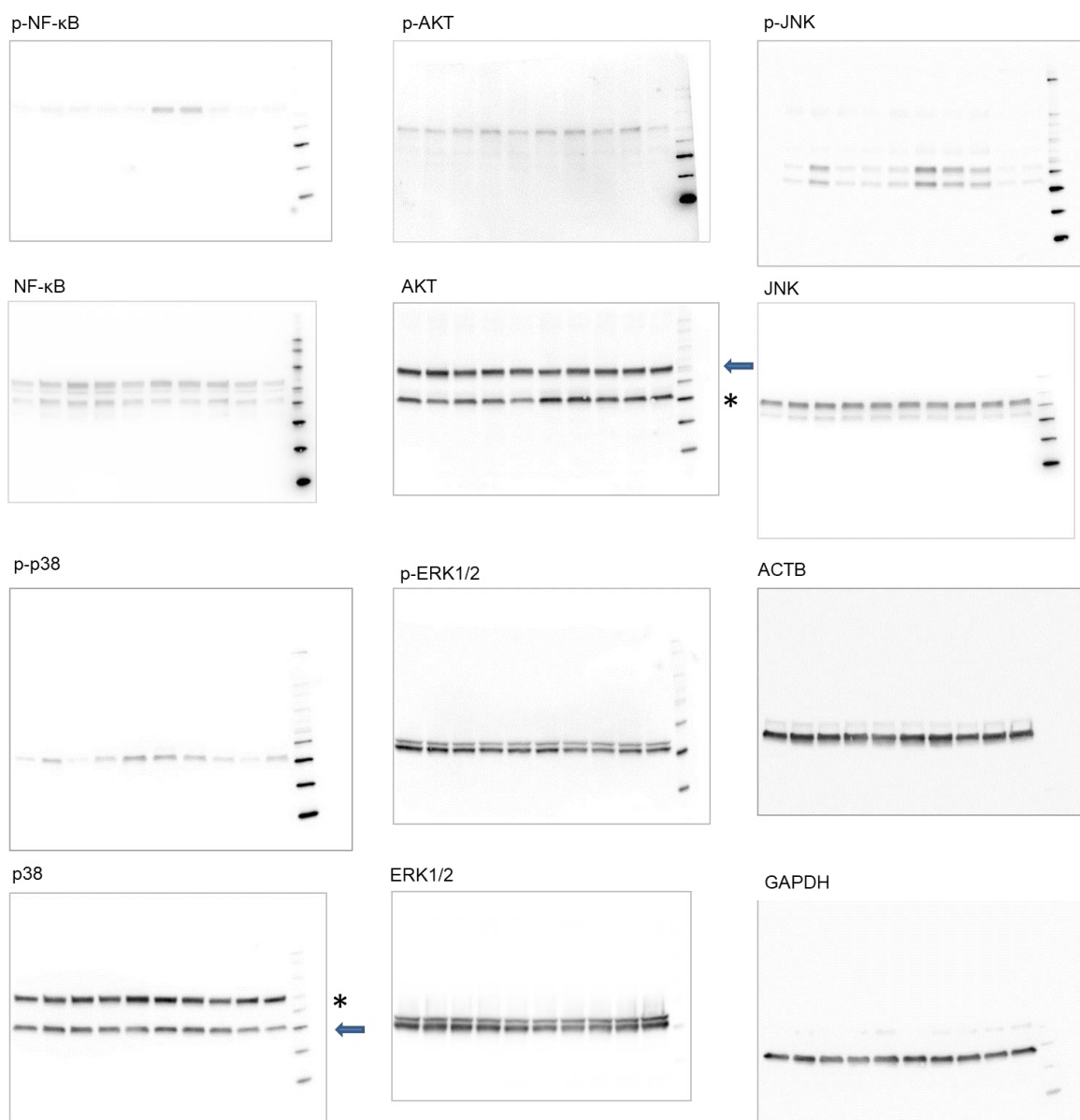


\* Sequential use of blots for staining of proteins with different size.

← band of interest

## Original western blots - Figure 1

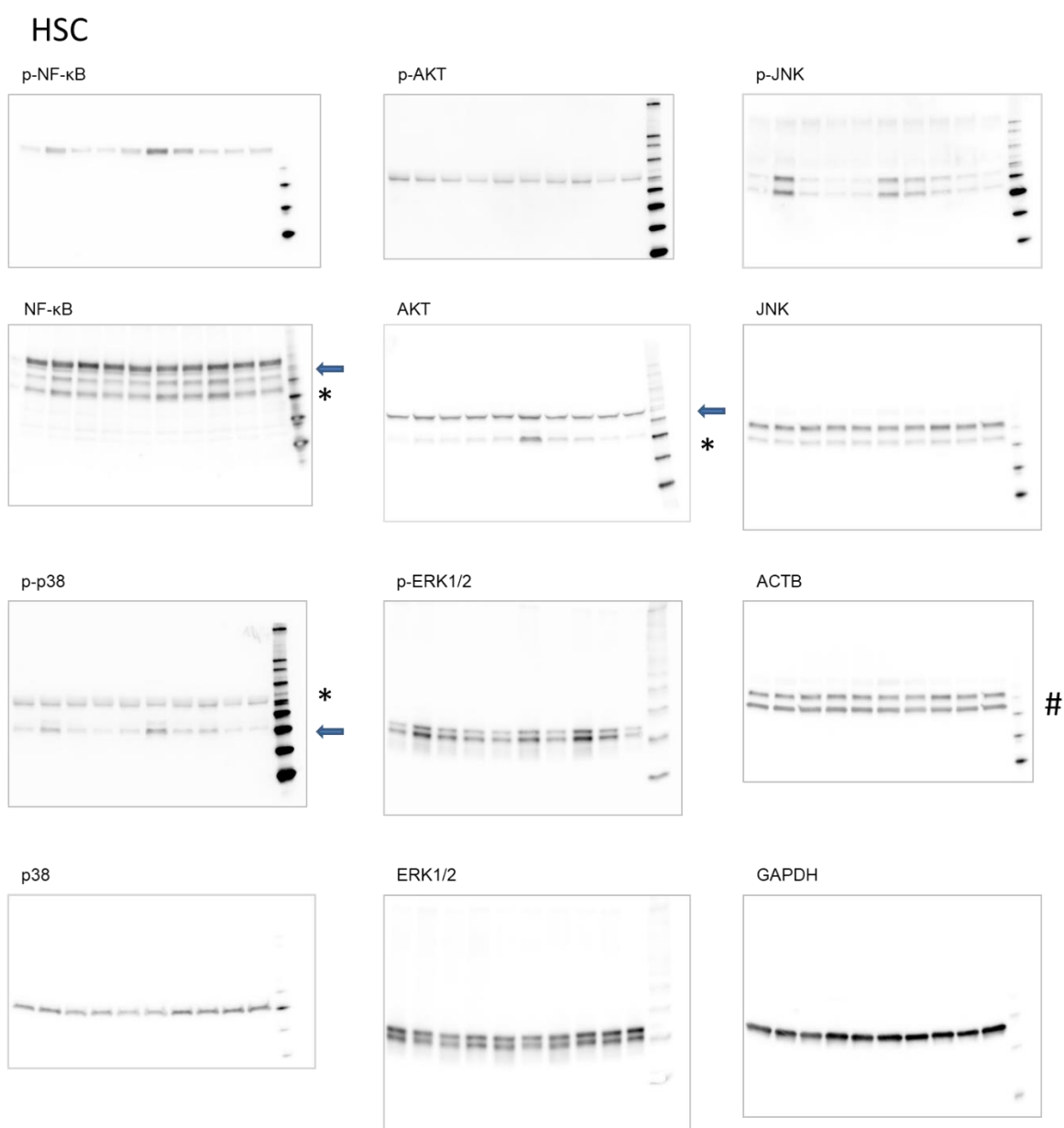
### LSEC



\* Sequential use of blots for staining of proteins with different size.

← band of interest

## Original western blots - Figure 1



\* Sequential use of blots for staining of proteins with different size.

← band of interest

# double band actin appeared in HSC



## Original western blots - Figure 2

