

SUPPLEMENTARY DATA

The analysis used data from 47 patients with human hepatocarcinoma (35 males and 12 females). The variables have been analyzed using the Statistical Package for Social Science (SPSS, version 22.0, Chicago, IL, USA).

1. Descriptive statistics

Sex	Number (%)	Mean survival (SD)
Male	35 (74.5)	35.76 (25.67)
Female	12 (25.5)	32.89 (26.72)
Total	47	35.03 (25.67)

Mean survival (all patients) is 35.03 months (SD 25.67)

Sex	eIF4A1 (SD)	eIF4A2 (SD)	DDX3 (SD)
Male	0.624 (0.495)	0.571 (0.406)	0.666 (0.699)
Female	0.660 (0.615)	0.541 (0.301)	0.330 (0.246)
Total	0.633 (0.522)	0.564 (0.379)	0.580 (0.630)

2. eIF4A1 in hepatocarcinoma

Low and high eIF4A1 protein levels were recoded into binary variables (0/1) using the median value (eIF4A1 = 0.523) as the cut-off. The whole dataset was then divided into 23 patients with eIF4A1 below the median, and 24 patients above the median. Statistical comparison between the two groups was performed using the **log-rank test**:

Marker	Number of patients (%)	Mean survival in months (SD)	Log-rank test
eIF4A1 < 0.523	23 (48.9)	45.68 (25.76)	–
eIF4A1 ≥ 0.523	24 (51.1)	24.82 (21.48)	0.005
Total	47	35.03 (25.67)	

Conclusion 1: **Patients with eIF4A1 above the median 0.523 survive on average shorter than patients with eIF4A1 below 0.523.**

3. eIF4A2 in hepatocarcinoma

Low and high eIF4A2 protein levels were recoded into binary variables (0/1) using the median value (eIF4A2 = 0.492) as the cut-off. The whole dataset was then divided into 23 patients with eIF4A2 below the median, and 24 patients above the median. Statistical comparison between the two groups was performed using the **log-rank test**:

Marker	Number of patients (%)	Mean survival in months (SD)	Log-rank test
eIF4A2 < 0.492	23 (48.9)	34.34 (23.78)	–
eIF4A2 ≥ 0.492	24 (51.1)	35.69 (27.86)	0.741
Total	47	35.03 (25.67)	

Conclusion 2: **Patients with eIF4A2 mRNA above the median 0.492 do not survive on average shorter than patients with eIF4A2 below 0.492.**

4. DDX3 in hepatocarcinoma

Low and high DDX3 protein levels were recoded into binary variables (0/1) using the median value (DDX3 = 0.433) as the cut-off. The whole dataset was then divided into 23 patients with DDX3 below the median, and 24 patients above the median. Statistical comparison between the two groups was performed using the **log-rank test**:

Marker	Number of patients (%)	Mean survival in months (SD)	Log-rank test
DDX3 < 0.433	23 (48.9)	27.99 (26.74)	–
DDX3 ≥ 0.433	24 (51.1)	41.77 (23.19)	0.142
Total	47	35.03 (25.67)	

Conclusion 3: **Patients with DDX3 above the median 0.433 survive on average longer than patients with DDX3 below 0.433 but without statistical significance.**

5. Survival analysis (univariate)

Variable	Survival (months) (SD)	p-value
<i>Age (years)</i>		
< 65	34.54 (24.90)	0.900
≥ 65	35.50 (26.92)	
<i>Sex</i>		
Female	35.76 (25.67)	0.742
Male	32.89 (26.72)	
<i>Cirrhosis</i>		
No	34.06 (18.19)	0.881
Yes	35.36 (28.00)	
<i>Etiology</i>		
HBV	31.54 (25.34)	0.245 [#]
HCV	32.33 (19.51)	
Ethanol	35.58 (31.40)	
<i>Size</i>		
< 3 cm	43.07 (26.38)	0.212
≥ 3 cm	32.27 (25.22)	
<i>Serum AFP</i>		
< 300 ng/ml	42.12 (30.19)	0.156
≥ 300 ng/ml	31.01 (22.29)	
<i>Tumor grade</i>		
II	42.23 (26.14)	0.137 [#]
III	38.17 (29.32)	
IV	23.85 (15.61)	
<i>eIF4A1</i>		
< 0.523	45.68 (25.76)	0.004
≥ 0.523	24.82 (21.48)	
<i>eIF4A2</i>		
< 0.492	34.34 (23.78)	0.860
≥ 0.492	35.69 (27.86)	
<i>DDX3</i>		
< 0.433	27.99 (26.74)	0.065

≥ 0.433	41.77 (23.19)	
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#One-way ANOVA

In the univariate analysis, a significant difference in survival was found only for the eIF4A1 protein.

6. Multivariate Cox regression analysis

A multivariate Cox proportional hazard model was constructed with survival as the outcome variable. The eIF4A1, eIF4A2, and DDX3 variables were included in three different models, together with all covariates (full model). Hazard ratios (HRs) and their 95% confidence intervals were calculated, and the Wald test was used for model testing.

Covariates	Full model (HR and 95% CI)	Full model (HR and 95% CI)	Full model (HR and 95% CI)
Age	1.016 (0.973–1.061)	1.025 (0.982–1.070)	1.019 (0.974–1.067)
Male sex	1.111 (0.450–2.744)	1.263 (0.537–2.970)	1.417 (0.613–3.275)
Cirrhosis (y/n)	1.229 (0.504–2.995)	0.770 (0.322–1.846)	0.856 (0.350–2.095)
Etiology			
HBV	Reference	Reference	Reference
HCV	1.077 (0.366–3.166)	0.594 (0.214–1.652)	0.733 (0.255–2.102)
Ethanol	1.289 (0.430–3.864)	0.889 (0.318–2.492)	1.551 (0.521–4.617)
Diameter > 3 cm	1.852 (0.804–4.265)	1.749 (0.758–4.038)	1.475 (0.672–3.240)
AFP > 300 ng/ml	3.107 (1.277–7.564) *	2.019 (0.841–4.848)	2.387 (0.977–5.834)
Grade			
II	Reference	Reference	Reference
III	0.500 (0.199–1.258)	0.983 (0.438–2.206)	1.017 (0.450–2.298)
IV	1.749 (0.712–4.295)	2.454 (1.048–5.751) *	2.790 (1.166–6.677) *
eIF4A1 ≥ 0.523 (median value)	4.751 (1.944–11.61) **	–	–
eIF4A2 ≥ 0.492 (median value)	–	1.110 (0.578–2.133)	–
DDX3 ≥ 0.433 (median value)	–	–	0.420 (0.201–0.876) *

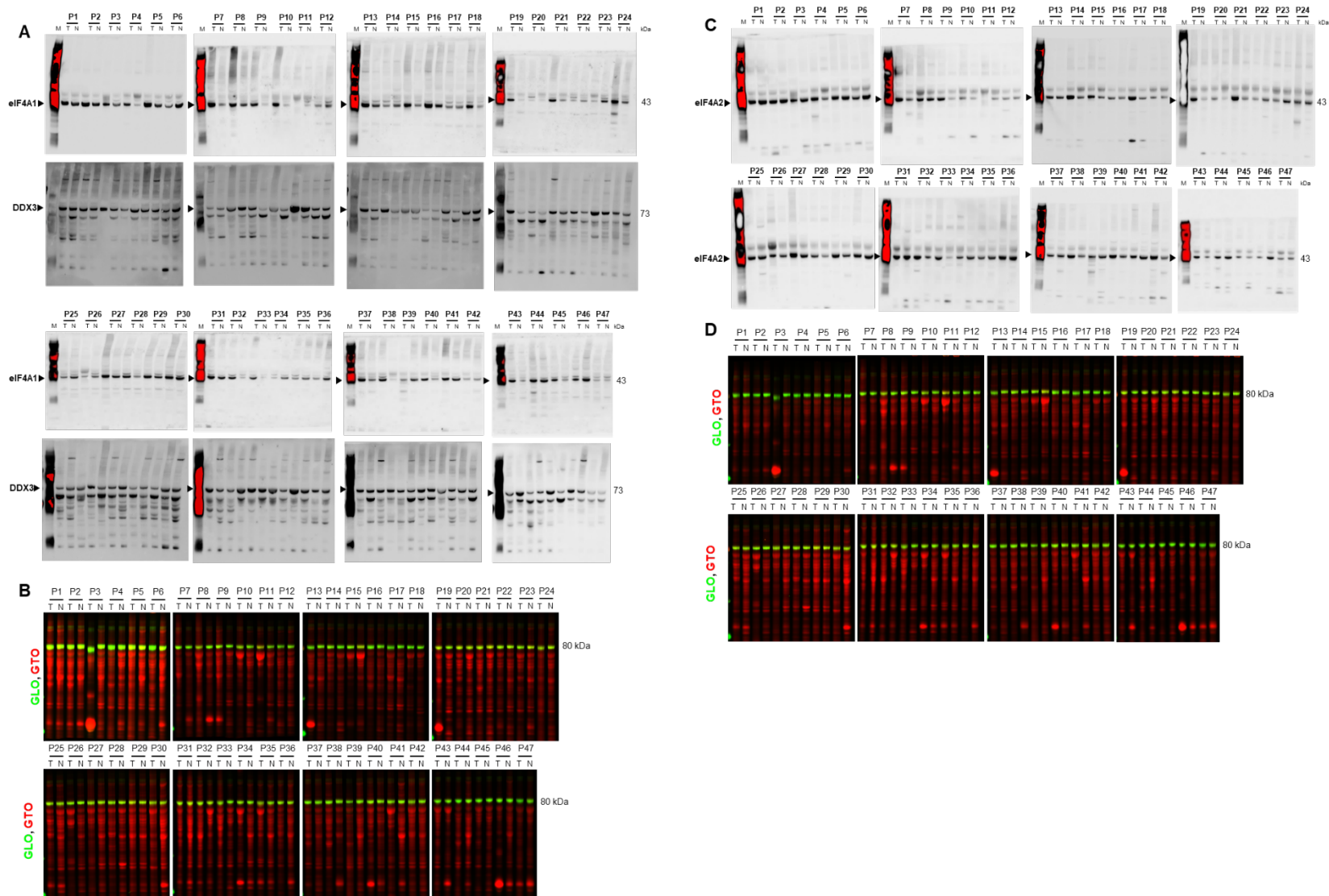
*p<0.05; **p<0.0001

Conclusion: **Levels of eIF4A1 protein greater than 0.523 are significant predictors of mortality in patients with hepatocellular carcinoma. Levels of DDX3 protein greater than 0.433 significantly predict more prolonged survival in patients with hepatocellular carcinoma.**

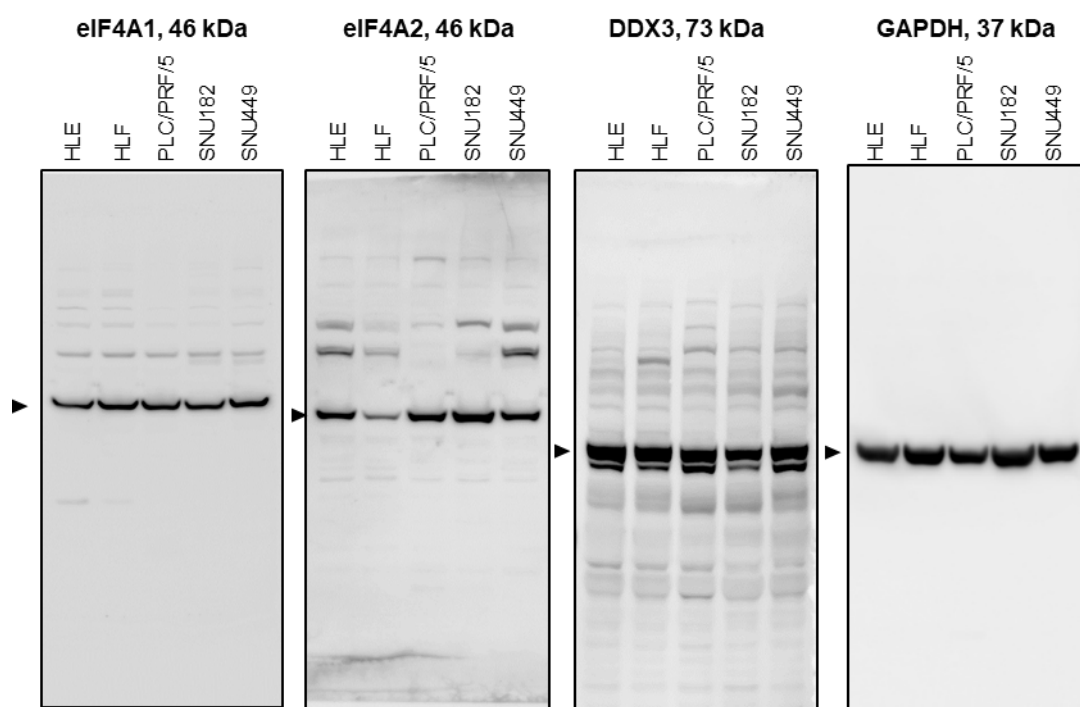
Supplementary Table S1. Clinicopathological features of HCC patients.

Variables	Features	
	HCCB	HCCP
Number of patients		
Male	17	18
Female	5	7
Age (Mean \pm SD)	64.77 \pm 8.7	66.32 \pm 10.6
Etiology		
HBV	9	9
HCV	7	11
Ethanol	3	3
N/A	3	2
Cirrhosis		
+	16	19
-	6	6
Tumor size		
> 5 cm	15	19
< 5 cm	7	6
Edmondson and Steiner grade		
I	0	0
II	7	6
III	11	9
IV	4	10
Serum alpha-fetoprotein level (ng/ml)		
> 300	13	17
< 300	9	8
Survival after partial liver resection (months)		
Means \pm SD	57.3 \pm 18.5	15.4 \pm 9.6

Abbreviations: N/A: Not applicable, SD: Standard deviation

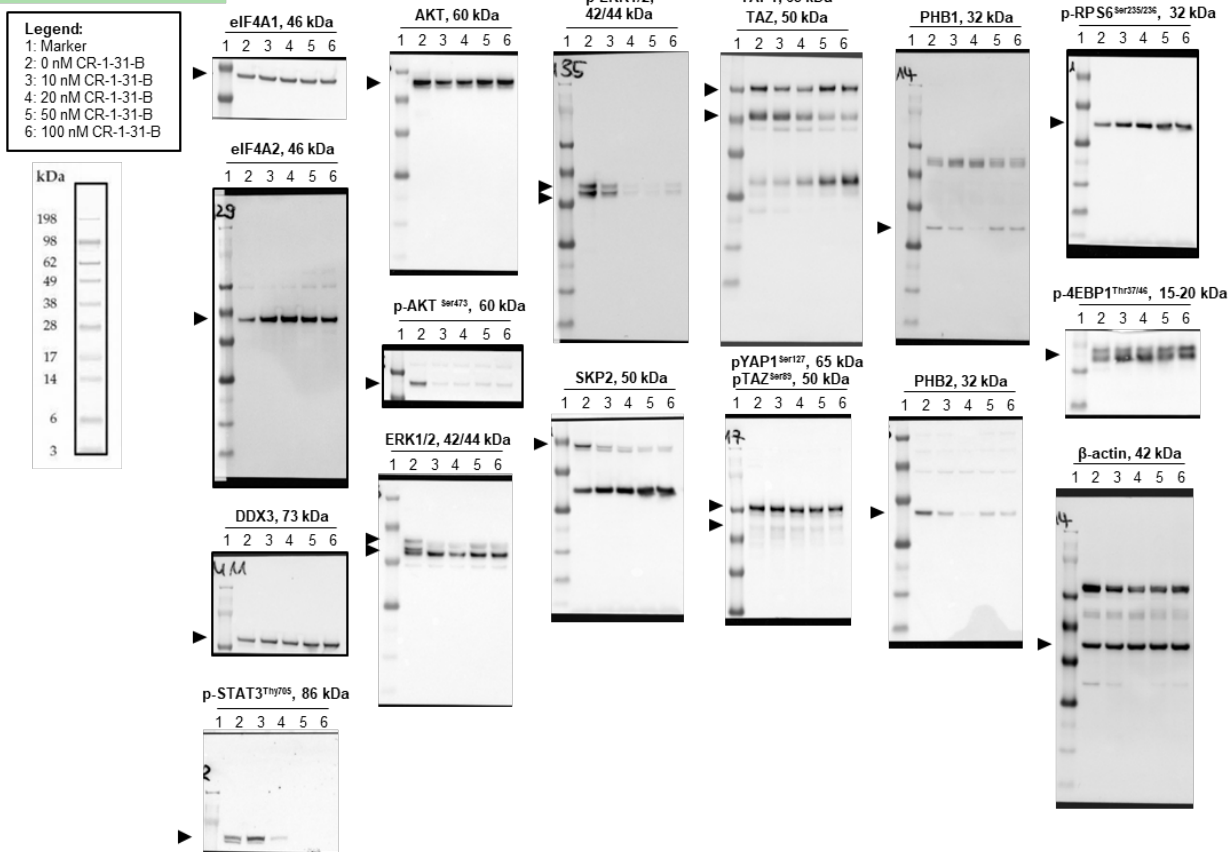


Supplementary Figure S1: Details of Western Blot data (uncropped membranes) in human HCC samples and paired non-tumor tissues. (a) Chemiluminescent images of eIF4A1 and DDX3 Western Blots. (b) Fluorescence images of eIF4A1 and DDX3 PAA gels with GLO (green) and GTO (red). (c) Chemiluminescent images of eIF4A2 Western Blots. (d) Fluorescence images of the eIF4A2 PAA gel with GLO (green) and GTO (red). N: Human normal tissue; T: Human HCC tumor tissue; P: Patient; GLO: Gel loading control at 80 kDa (green); GTO: Total protein signals (red).

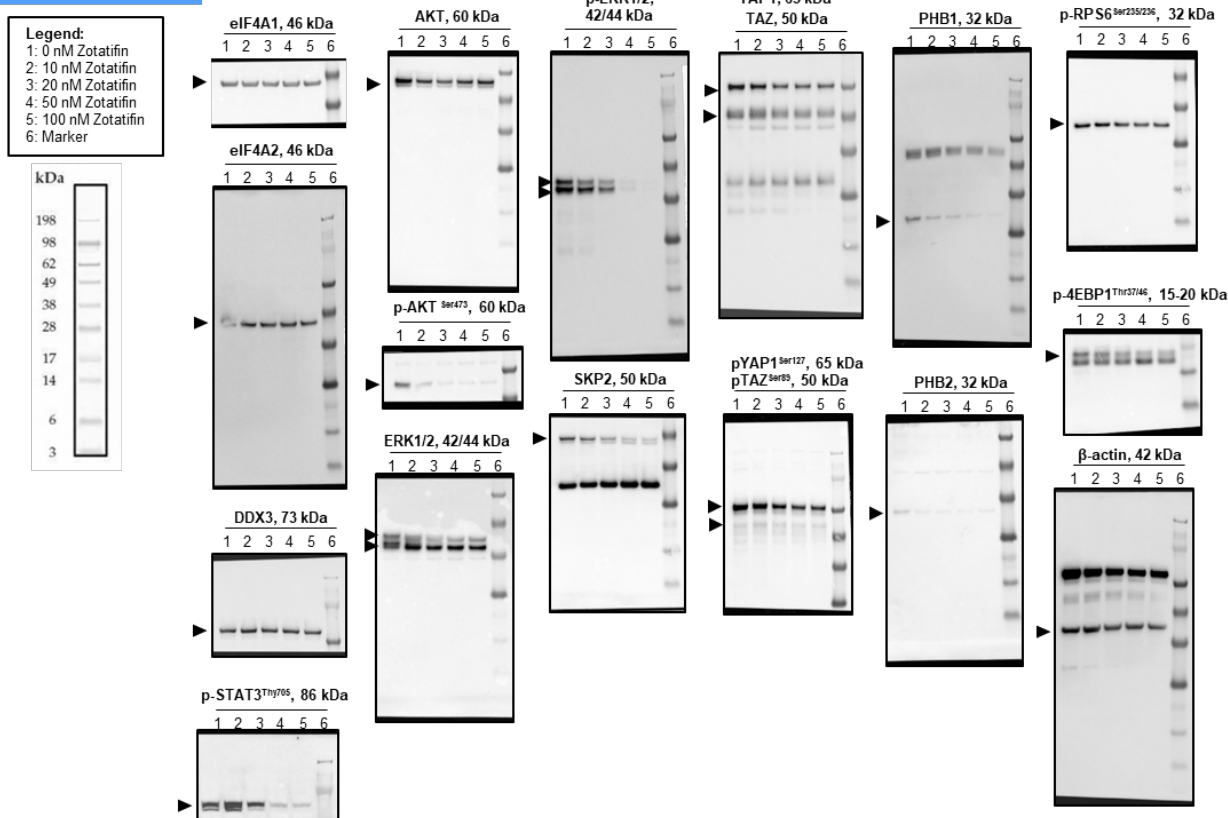


Supplementary Figure S2: Detailed Western Blot images (uncropped membranes) of eIF4A1, eIF4A2, DDX3, and GAPDH expression in different cell lines.

A HLE + CR-1-31-B:

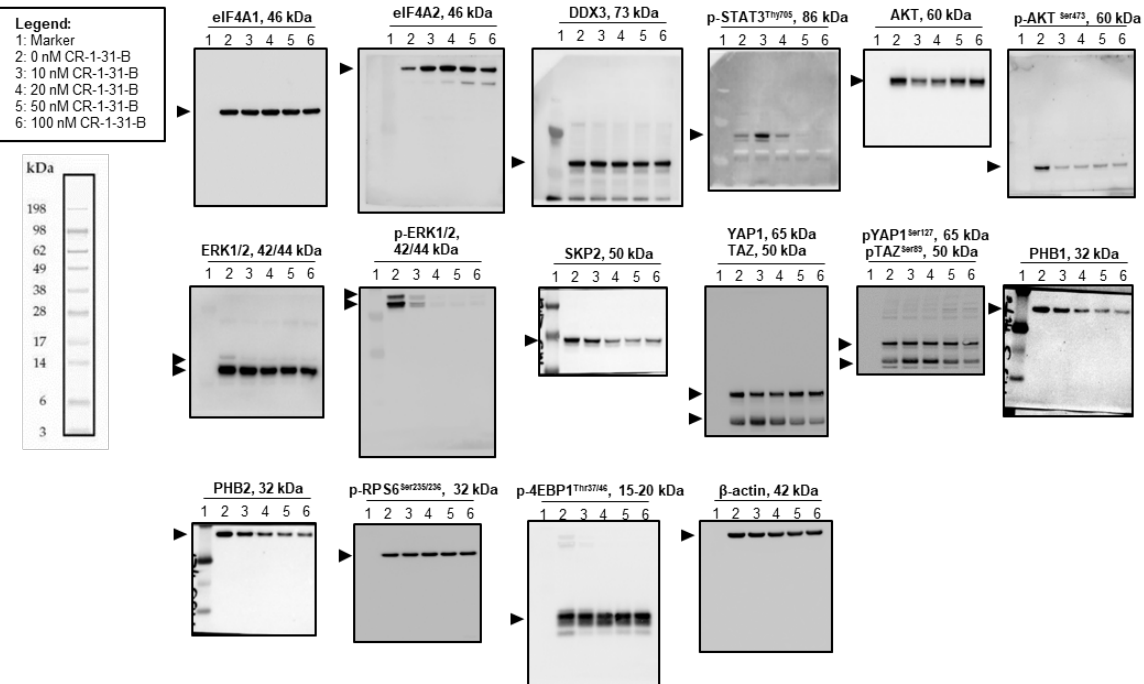


B HLE + Zotatfin:

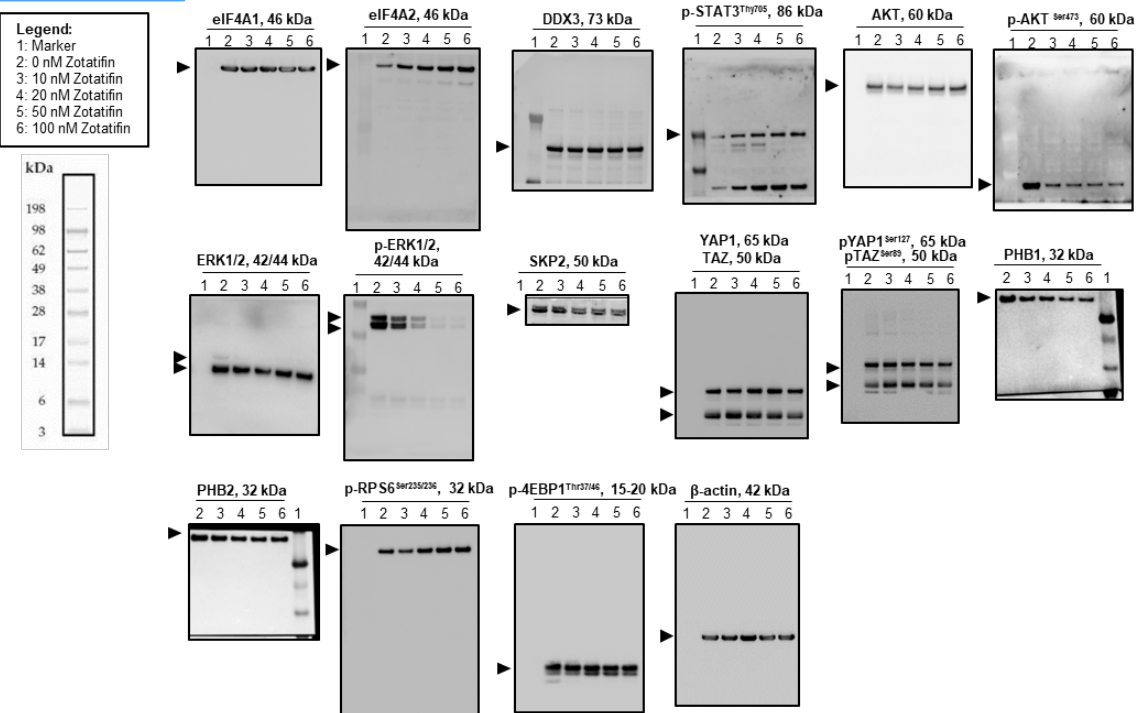


Supplementary Figure S3: Details of Western Blot data (uncropped membranes) in HLE cells.

A HLF + CR-1-31-B:



B HLF + Zotatifin:

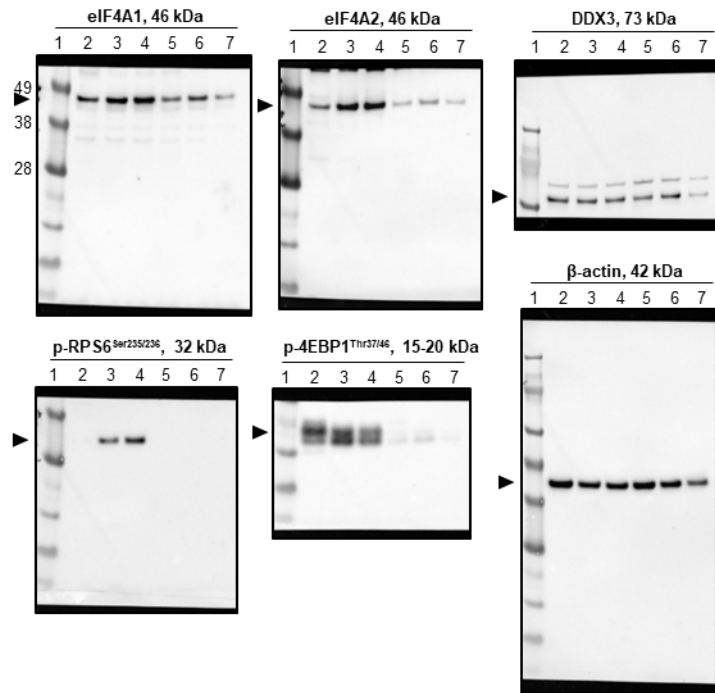
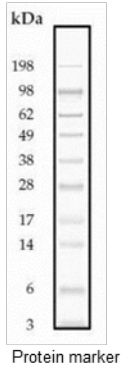


Supplementary Figure S4: Details of Western Blot data (uncropped membranes) in HLF cells.

A HLE

Legend:

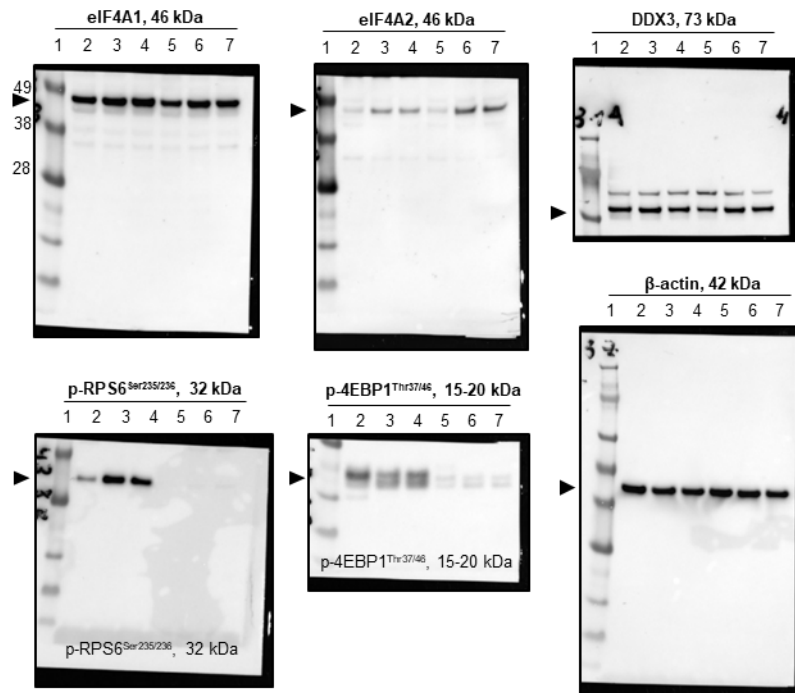
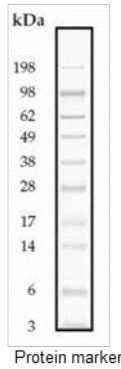
1. Marker
2. DMSO
- 3: 10 nM CR-1-31-B
- 4: 10 nM Zotatfin
- 5: 10 nM Rapalink1
- 6: 10 nM CR-1-31-B + 10 nM Rapalink1
- 7: 10 nM Zotatfin + 10 nM Rapalink1



B HLF

Legend:

1. Marker
2. DMSO
- 3: 10 nM CR-1-31-B
- 4: 10 nM Zotatfin
- 5: 10 nM Rapalink1
- 6: 10 nM CR-1-31-B + 10 nM Rapalink1
- 7: 10 nM Zotatfin + 10 nM Rapalink1



Supplementary Figure S5: Details of Western Blot data (uncropped membranes) in HLE and HLF cells.