Supplementary Materials and Methods:

Plasmid Design

HuR cDNA was cloned into pGEX-6P1 backbone (Amersham) using EcoRI and XhoI sites. This construct was used for the RNA pull down assays.

To generate the 4E3'UTR construct, the 3'UTR of eIF4E was amplified from DNAse treated RNA isolated from HEK293 or U2Os cells using Titan-PCR kit. The PCR product was re-amplified using primers with EcoRI and XhoI restriction sites and subsequently cloned after the LacZ ORF in pcDNA3.1HisLacZ. According to sequencing results, the construct corresponds to bases 1506-2476 of eIF4E (gi: 54873625). Shorter constructs of the 4E3'UTR corresponding to regions with AU-rich sequences (1^{st}+2^{nd}+3^{rd} ARE, 1^{st} ARE, 2^{nd} ARE, 3^{rd} ARE) and without (noARE) were cloned using the same strategy as above. pfuTurbo (Stratagene) was used in all PCR reactions and the primers used to generate all of the above constructs are listed in Table 1.

T7 RNA probes

Templates for *in vitro* synthesis of biotinylated transcripts were generated by PCR from either human eIF4E cDNA (Open Biosystems; gi:23243253), pcDNA3.1HisLacZ/ 4E3'UTR, or 3'UTR-GAPDH using primers listed in Table 2. All 5' primers contained the T7 polymerase sequence. *In vitro* transcriptions were carried out using Megascript kit (Ambion) according to the manufacturer's instruction, using 1:10 ratio of CTP and 11-biotin CTP (NEB). *In vitro* transcribed, biotinylated probes were purified using

phenol:chlorophorm extraction and MegaClean columns (Ambion), and the efficiency of biotinylation was verified by Northern Blot (data not shown).

Table 1: List of primers used for construct design

Construct	Primer Name	Primer Sequence	DNA sequence (bp)
pGEX6P1-HuR	Eco-HuR-5F	GCACATGAATTCATGTCTAATGGTTATG	1-981[a]
	Xho-HuR-3R	CAGTGACTCGAGTTATTTGTGGGACTTG	
pcDNA3.1LacZ-4E3'UTR	IT-Eco-4E3UTR-F	GCACATGAATTCTTAAGAAGACAC	1506-2476 [b]
	IT-Xho-4E3UTR-R	CAGTGACTCGAGTAAAAGACAATT	
pcDNA3.1LacZ-noARE	IT-Eco-4E3UTR-F	GCACATGAATTCTTAAGAAGACAC	1506-2238 [b]
	NS-Xho-REV1	CAGTGACTCGAGATCACTGATTTGAAT	
pcDNA3.1LacZ-1^{st}+2^{nd}+3^{rd} ARE	NS-Eco-4EUTR4-F	GCACATGAATTCATTCAAATCAGTGAT	2224-2476 [b]
	IT-Xho-4E3UTR-R	CAGTGACTCGAGTAAAAGACAATT	
pcDNA3.1LacZ-1^{st} ARE	1st-ARE-Eco-5F	GCACATGAATTCATTTATGCATTTCAT	2211-2283 [b]
	1st-ARE-Xho-3R	CAGTGACTCGAGTTTTTTATAATCCAC	
pcDNA3.1LacZ-2^{nd} ARE	2nd-ARE-Eco-5F	GCACATGAATTCCCTAACTAGAATTAG	2304-2376 [b]
	2nd-ARE-Xho-3R	CAGTGACTCGAGAATAACCTAAGTAAT	
pcDNA3.1LacZ-3^{rd} ARE	3rd-ARE-Eco-5F	GCACATGAATTCTTTTAACACTTTGTA	2348-2420 [b]
	3rd-ARE-Xho-3R	CAGTGACTCGAGGAATGGGACTGCTTT	

* Restriction sites are underlined

a) h-ELAV/HuR; gi:38201713; 1-981 bp

b) h-eIF4E; gi:54873625; 1-2493 bp

Table 2: List of primers used to generate T7 RNA probes

T7-Probe	Name	Primer Sequence	DNA sequence
4E-5'UTR	T7-5UTR-5F	TAATACGACTCACTATAGGGAGACGGGGCCCGGAGTGGCTT	1-855 [a]
	T7-5UTR-3R	GGAGCGGTTGTGCGATCAGATCGATCTAAG	
4E-CDR	T7-eIF4E-5F	TAATACGACTCACTATAGGGAGAATGGCGACTGTCGAA	856-1509 [a]
	T7-eIF4E-3R	TTAAACAACAAACCTATTTTTAGTGGT	
4E-3'UTR	T7-4EUTR1	TAATACGACTCACTATAGGGAGATTAAGAAGACACCTTCTG	1506-2476 [a]
	T7-3UTR-REV	AAGACAATTCACTGTACACATTTTATT	
	T7-GAPDH-R1	GGTTGAGCACAGGGTACTTTATTGATG	
1st ARE	T7-ARE1-5F	TAATACGACTCACTATAGGGAGAATTTATGCATTTCAT	2211-2283 [a]
	T7ARE1-REV	TTTTTTATAATCCACAATTATGTT	
2nd ARE	T7-ARE2-5F	TAATACGACTCACTATAGGGAGACCTAACTAGAATTAG	2304-2376 [a]
	T7-ARE2-REV	AATAACCTAAGTAATACAAAGTGT	
3rd ARE	T7-ARE3-5F	TAATACGACTCACTATAGGGAGATTTTAACACTTTGTA	2348-2420 [a]
	T7-ARE3-REV	GAATGGGACTGCTTTTCTACTTGA	
GAPDH-3'UTR	T7-GAPDH-F1	TAATACGACTCACTATAGGGAGAGACCCCTGGACCACC	1111-1310 [b]

*T7 polymerase sequences are underlined

a) h-eIF4E; gi:54873625; 1-2493 bp

b) h-GAPDH; gi:83641890; 1-1310 bp