The mRNA encoding the JUND tumor suppressor detains nuclear RNA-binding proteins to assemble polysomes that are unaffected by mTOR

Gatikrushna Singh[1], Sarah E. Fritz[2,#], Bradley Seufzer[1] Kathleen Boris-Lawrie[1,2*]

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I. Figure and Figure Legends S1-S4

Figure S1

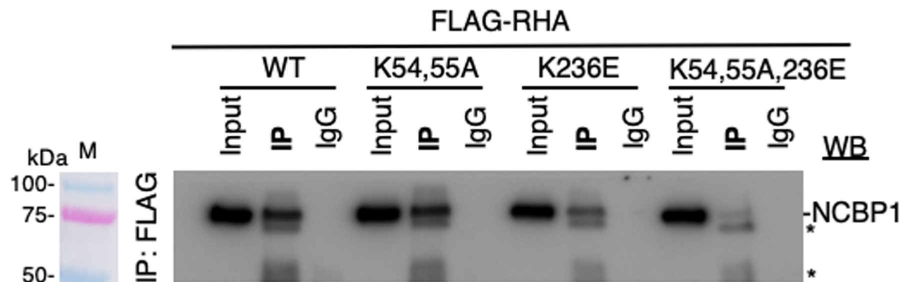

Figure S1. FLAG-RHA interaction with NCBP1 (prolonged exposure)
The prolonged exposure of the WB shown in Fig. 4C identifies NCBP1 in the FLAG-immunoprecipitates. The antiserum detected the specific proteins on the immunoblots, as shown relative to the pre-stained molecular mass markers (M). The same image of the molecular mass markers was used for each panel.

Figure S2

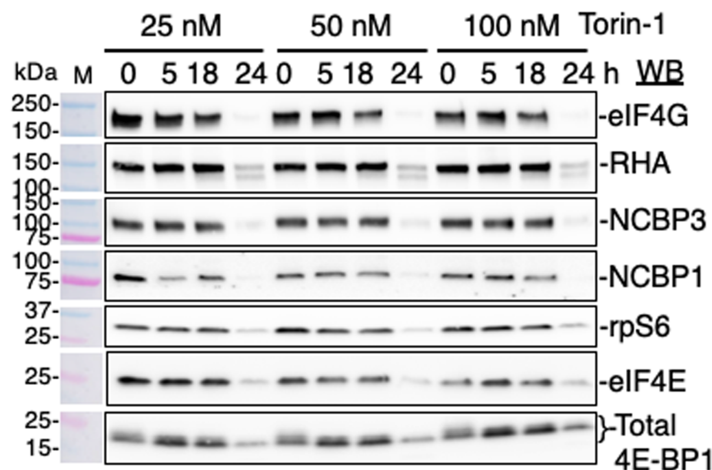

Figure S2. Standardization of the dose and the incubation period for activation of 4E-BP1 by Torin-1
HEK293 cells were exposed to 0, 25, 50 or 100 nM mTOR inhibitor Torin-1 for 0, 5 18 or 24 h. The cells were washed once with ice-cold 1× PBS and lysed in RIPA buffer. The cell lysates (20 μg) were immunoblotted with the indicated antisera. The antiserum detected the specific proteins on the immunoblots, as shown relative to the pre-stained molecular mass markers (M). The same image of the molecular mass markers was used for each panel.

Figure S3

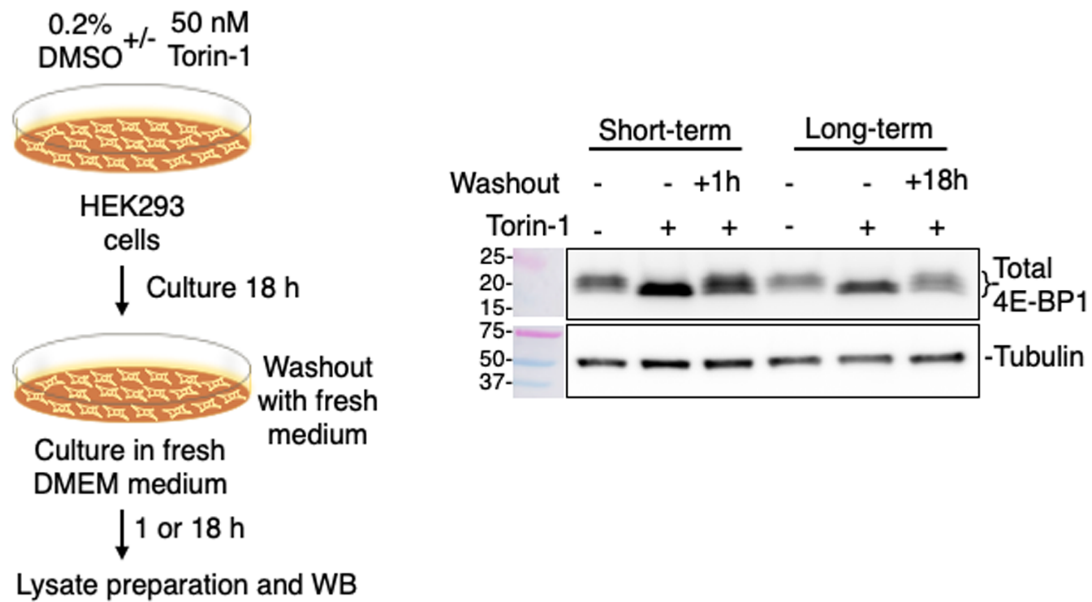

Figure S3. Demonstration of 4E-BP1 levels before and after Torin-1 treatment
The cartoon on the left outlines the protocol of Torin-1 treatment and washout. HEK293 cells treated with 50 nM Torin-1 for 18 h and the washed with fresh medium to eliminate Torin-1. After 1 or 18 h in Torin-1 free medium, cells were collected and lysates were analyzed by WB with the indicated antisera. The antiserum detected the specific proteins on the immunoblots, as shown relative to the pre-stained molecular mass markers (M). The same image of the molecular mass markers was used for each panel.

Figure S4

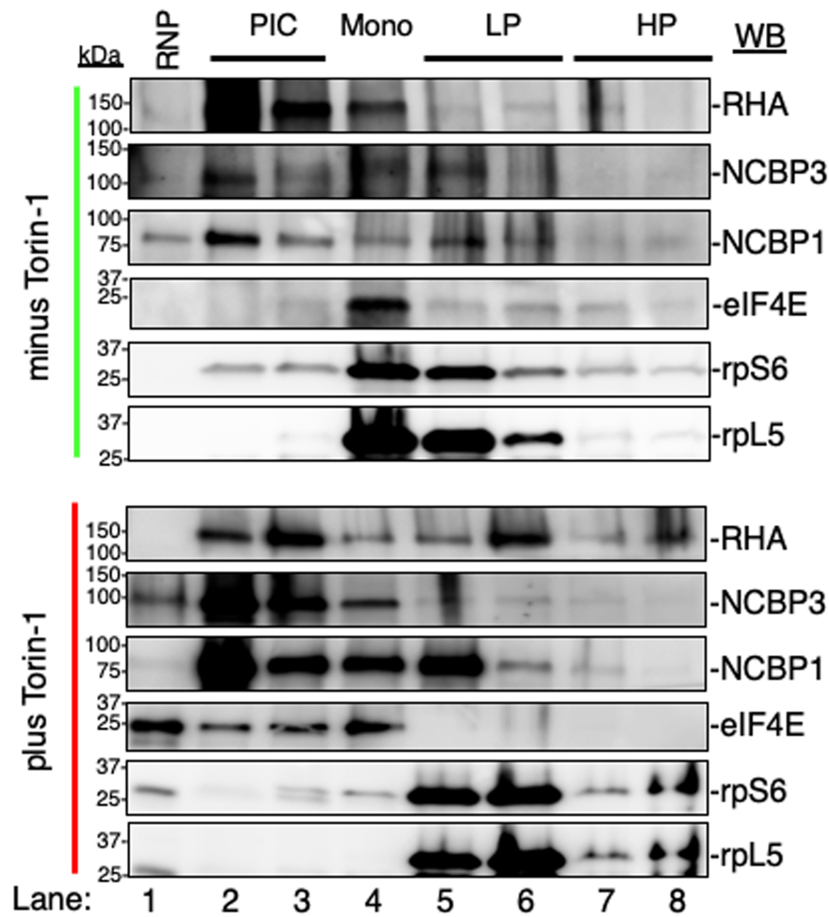

Figure S4. The distribution of proteins in fractions of sucrose density gradients
The HEK293 cells treated minus or plus 50 nM Torin-1 for 18 h were lysed and cyto lysates were fractionated on sucrose gradients. Proteins in the gradient fractions were precipitated and analyzed by WB with the indicated antiserum. Lane 1: ribonucleoprotein (RNP); lane 2 and 3: pre-initiation complex (PIC); lane 4: monosome (mono); lane 5 and 6: light polysomes (LP); lane 7 and 8: heavy polysomes (HP). The antiserum detected the specific proteins on the immunoblots, as shown relative to the pre-stained molecular mass markers (M). The same image of the molecular mass markers was used for each panel.

II. Table and Table Legends S1-S4

Table S1

Table S1 is a spreadsheet.

Table S1. Densitometry quantification of WBs
The WBs were quantified by ImageJ and the densitometry of the individual bands has been presented according to the layout of the corresponding figure. Red font represents positive co-IP. The ratio of the immunoprecipitated protein was calculated relative to the input lysate.

Table S2

Immunoprecipitation		
Antibody	**Source**	**Catalog No.**
DHX9/RHA	Vaxron	PA-001
DDX3	Bethyl	A300-474A
NCBP1	Bethyl	A301-794A
NCBP2	Proteintech	11950-I-AP
	Bethyl	A302-553AT
NCBP3	Proteintech	24384-I-AP
eIF4E	Santa Cruz	SC-9976AC
	Sigma	E5906
eIF4G	Cell Signaling	#2498S
FLAG	Sigma	F7425
	Agilent	200473
	Sigma	F1804

Table S2. Immunoprecipitation antibodies
These antibodies were used for immunoprecipitation of the candidate proteins.

Table S3

Western blot			
Antibody	**Source**	**Catalog No.**	**Dilution**
DHX9/RHA	Vaxron	PA-001	1:7500
	Sigma	WH0001660M1	1:1000
DDX3	Bethyl	A300-474A	1:1000
	Santa Cruz	SC-81247	
NCBP1	Bethyl	A301-793A	1:1000
	Cell Signaling	D72227	
	Santa Cruz	SC-271304	
NCBP2	Proteintech	11950-I-AP	1:200
	Santa Cruz	SC-137123	1:100
	Bethyl	A302-553AT	1:500
	Sigma	SAB4500912	1:500
NCBP3	Proteintech	24384-I-AP	1:1000
eIF4E	Santa Cruz	SC-9976	1:1000
	Sigma	E5906	
	Cell Signaling	#9742S	
eIF4G	Cell Signaling	#2498S	1:1000
4E-BP1	Cell Signaling	#9452S	1: 500
		#9644S	
PABPC1	Abcam	ab6125	1:1000
rpL5	Bethyl	A303-933A	1:1000
rpS6	Cell Signaling	#2217	1:1000
JUND	Santa Cruz	SC-271938	1:500
FLAG	Sigma	F7425	1:5000
	Sigma	F1804	
	Agilent	200473	1:1000
GAPDH	Abcam	ab-181602	1:1000
	Santa Cruz	SC-47724	1:500
	Proteintech	10494-I AP	1:1000
		60004-I-Ig	
Tubulin	Santa Cruz	SC-23948	1:1000

Table S3. Western blot antibodies
These antibodies were used for WB of the candidate proteins.

Table S4

Name	Gene	Sequence
Quantitative real time PCR primer pairs		
KB1628	junD sense	5’ GCC TGG AGG AGA AAG TGA AG 3’
KB1629	junD antisense	5’ GGC TGA GGA CTT TCT GCT TG 3’
KB2320	ncbp3 sense	5’ GCA GGA AGA CAG TTC AGA TG 3’
KB2321	ncbp3 antisense	5’ ACT TCT TCT GGC TGC TCC AA 3’
KB2326	rha sense	5’ GCC AGA GAC TTT GTT AAC TAT 3’
KB2327	rha antisense	5’ CAT TTG CTG TAG TGT CAG GAG 3’
KB1371	gapdh sense	5’ CAT CAA TGA CCC CTT CAT TGA C 3’
KB1372	gapdh antisense	5’ CGC CCC ACT TGA TTT TGG A 3’
KB2399	cjun sense	5’ TGA CTG CAA AGA TGG AAA CGA CCT TC 3’
KB2400	cjun antisense	5’ CAG GGT CAT GCT CTG TTT CAG GAT C 3’

Table S4. Oligonucleotide sequence used to identify candidate genes
The oligonucleotides were synthesized from integrated DNA technology (IDT) and used in RT-qPCR for quantification of the candidate genes.