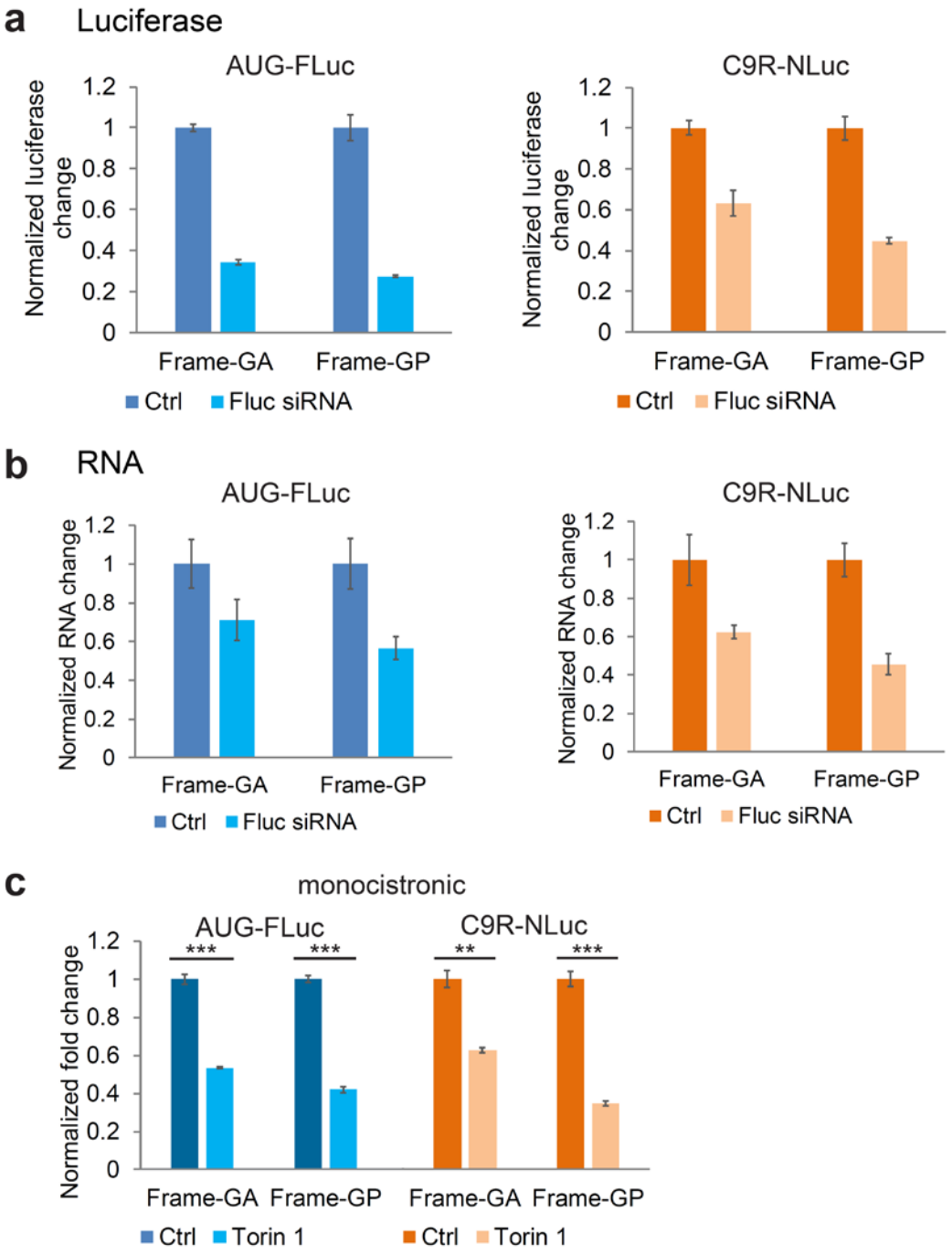
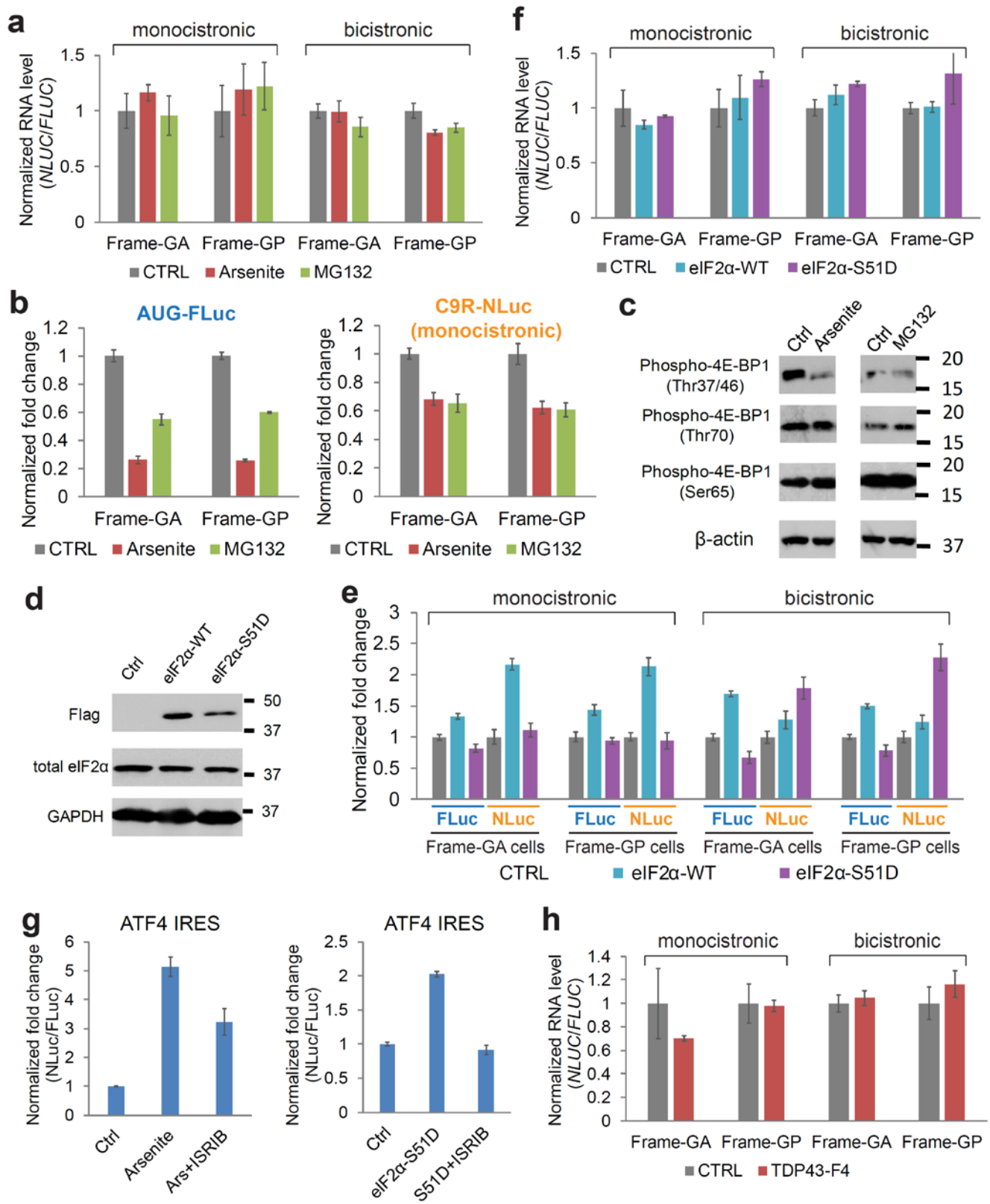
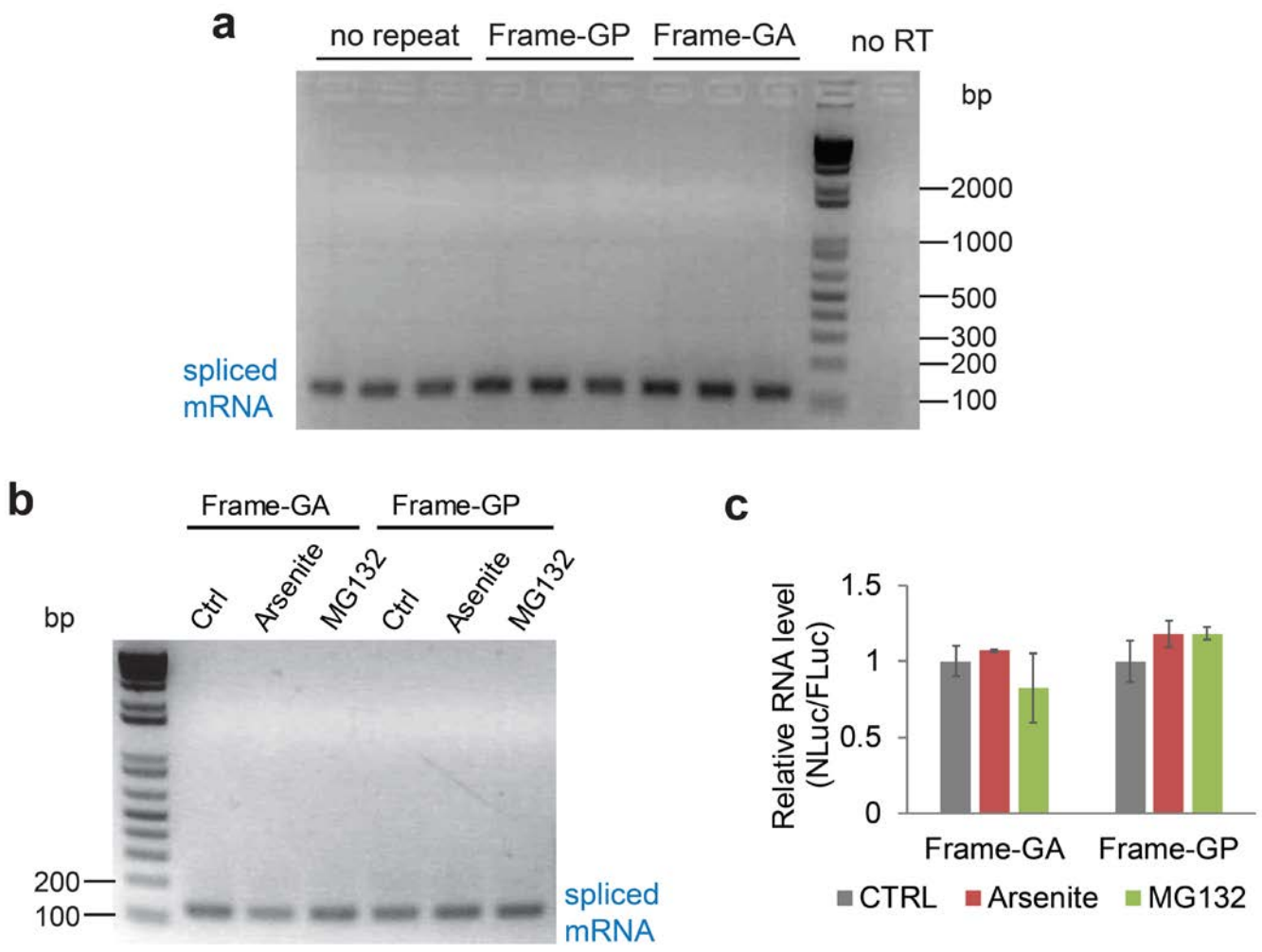


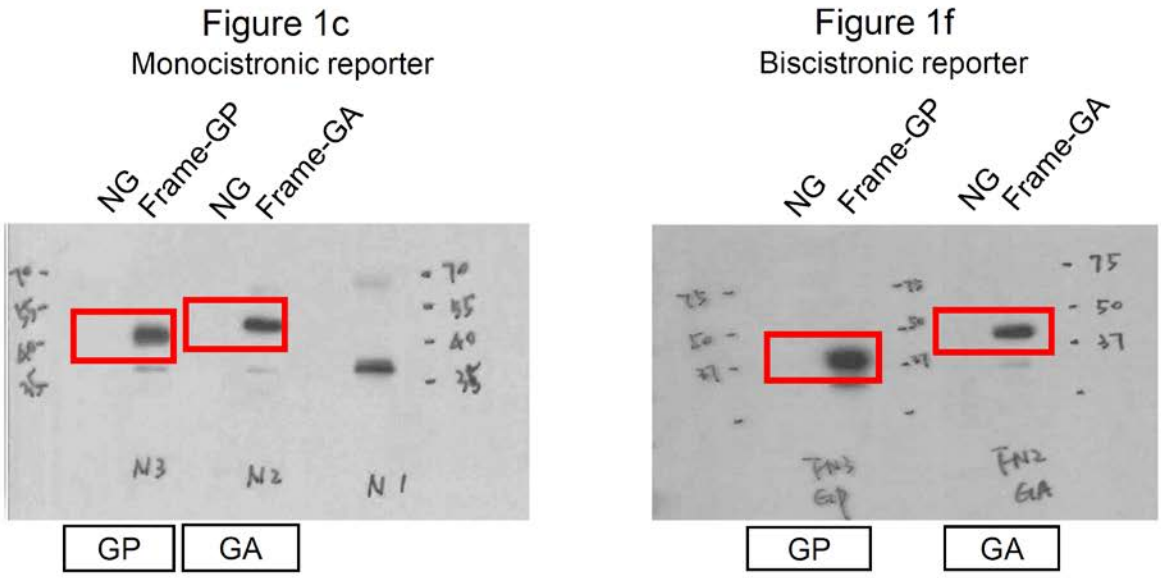
Supplementary Figure 1: Dual-luciferase reporters for RAN translation of C9ORF72 GGGGCC repeats. (a) Relative luciferase levels of AUG-FLuc (left) and C9R-NLuc (right) in the bicistronic reporter cell lines in presence of non-targeting siRNA or siRNA against Fluc. Data are mean ± s.e.m. from three biological replicates. **(b)** Relative RNA levels of FLuc (left) and NLuc (right) in the bicistronic reporter cells as above, revealed by qRT-PCR. Data are mean ± s.e.m. from three biological replicates. **(c)** Expression of AUG-FLuc and C9R-NLuc in monocistronic reporter cells under mTOR pathway inhibition by Torin 1 treatment. Data are mean ± s.e.m. from three biological replicates. ***P*<0.005, ****P*<0.0005, two-tailed *t* test.

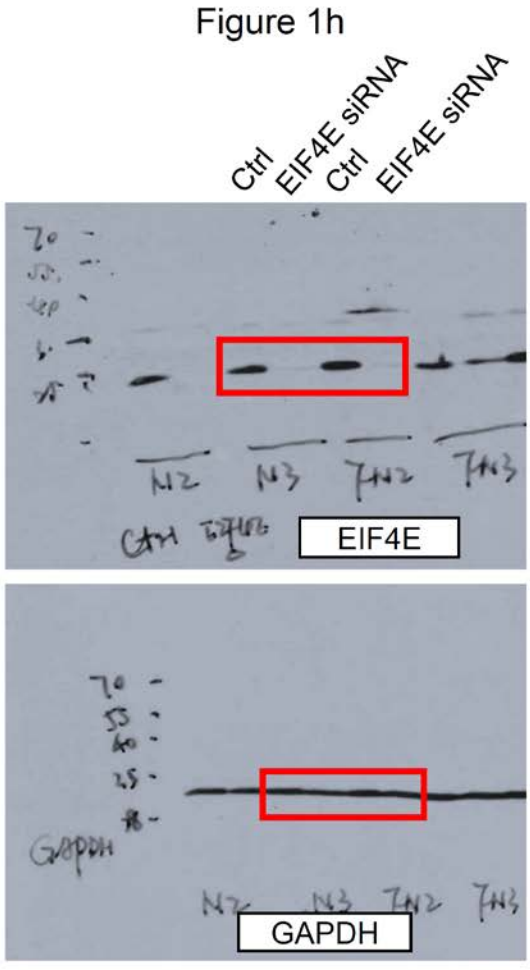

a
monocistronic
bicistronic
Normalized RNA level (NLUC/FLUC)
Frame-GA
Frame-GP
CTRL
Arsenite
MG132
f
eIF2α-WT
eIF2α-S51D
b
AUG-FLuc
C9R-NLuc (monocistronic)
Normalized fold change
c
Ctrl
Arsenite
MG132
Phospho-4E-BP1 (Thr37/46)
Phospho-4E-BP1 (Thr70)
Phospho-4E-BP1 (Ser65)
β-actin
d
eIF2α-WT
eIF2α-S51D
Flag
total eIF2α
GAPDH
e
FLuc
NLuc
Frame-GA cells
Frame-GP cells
g
ATF4 IRES
Normalized fold change (NLuc/FLuc)
Ars+ISRIB
S51D+ISRIB
h
TDP43-F4

Supplementary Figure 2: eIF2α phosphorylation enhances cap-independent RAN translation under stress. (a) NLuc and FLuc RNA levels were measured by qRT-PCR without and with arsenite or MG132 stress stimuli in each reporter cell line. NLuc was normalized to FLuc levels. Error bars represent s.e.m in four biological and technical replicates. **(b)** Expression of monocistronic luciferase reporters under treatment with arsenite or MG132 stimuli. Relative expression of AUG-FLuc and C9R-NLuc reporters were compared with no stress control. The luciferase signals were normalized to the total protein amount. Data are mean ± s.e.m. from three biological replicates. **(c)** Reporter cells were treated with arsenite or MG132. Immunoblotting of phospho-4E-BP1 using antibodies recognizing different phosphorylation sites. β-actin was blotted as internal control. **(d)** Reporter cells were transfected with plasmids expressing GFP (negative control), FLAG-tagged wild type or S51D mutant of eIF2α. The relative expression levels were examined by immunoblotting using antibodies recognizing the FLAG tag and total eIF2α. GAPDH was blotted as internal control. **(e)** After 1 day of transfection, cells were induced to express translation reporters by doxycycline and luciferase activities were measured after another 24 hours. Relative expression of AUG-FLuc and C9R-NLuc reporters were compared with GFP transfection control. The luciferase signals were normalized to the total protein amount. Data are mean ± s.e.m. from three biological replicates. **(f)** NLuc and FLuc RNA levels were measured by qRT-PCR after transfection of GFP, wild type or S51D mutant of eIF2α in each reporter cell line. **(g)** Fold change of ATF4 5'UTR IRES-mediated translation with arsenite stimuli (left) or eIF2α S51D expression (right), without or with pre-treatment of ISRIB. Error bars represent s.e.m in three biological replicates. **(h)** NLuc and FLuc RNA levels were measured by qRT-PCR after transfection of GFP (negative control), or TDP43-F4 in each reporter cell line. Error bars represent s.e.m in three biological replicates.



Supplementary Figure 3: RNA splicing and expression of the RAN translation reporter for intronic GGGGCC repeats. (a) PCR amplification of spliced mRNA from the reporters with and without the expanded repeats in the intron. **(b)** PCR amplification of spliced reporter mRNA upon stress stimuli. **(c)** The relative level of NLuc normalized to FLuc is not changed by stress stimuli, measured by qRT-PCR in each reporter cell line. Error bars represent s.e.m in three biological replicates.





Supplementary Figure 4 (cont'd): Uncropped Western Blots shown in main figures.

Figure 1j

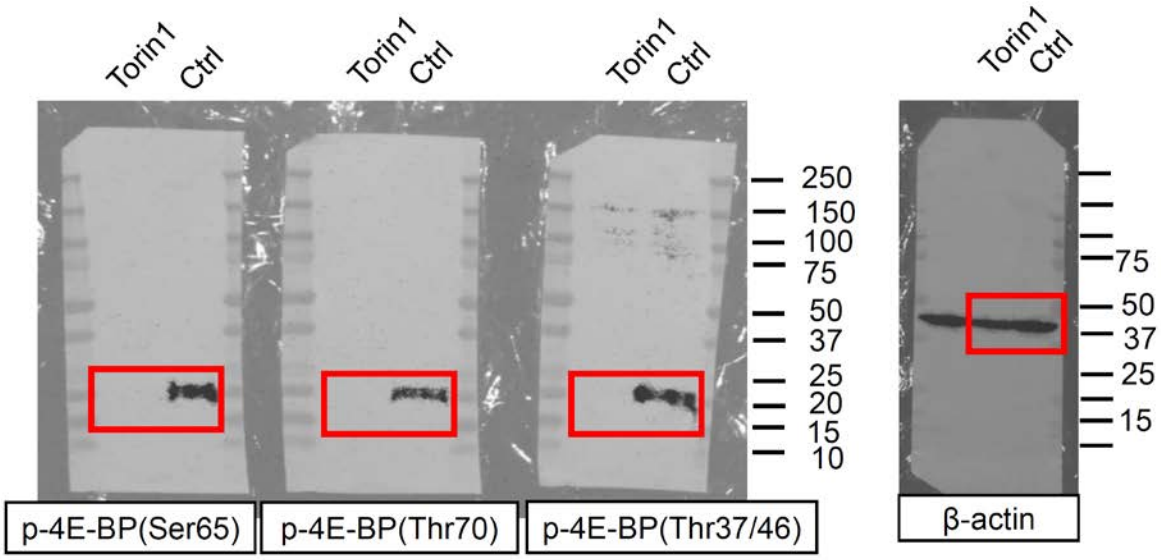
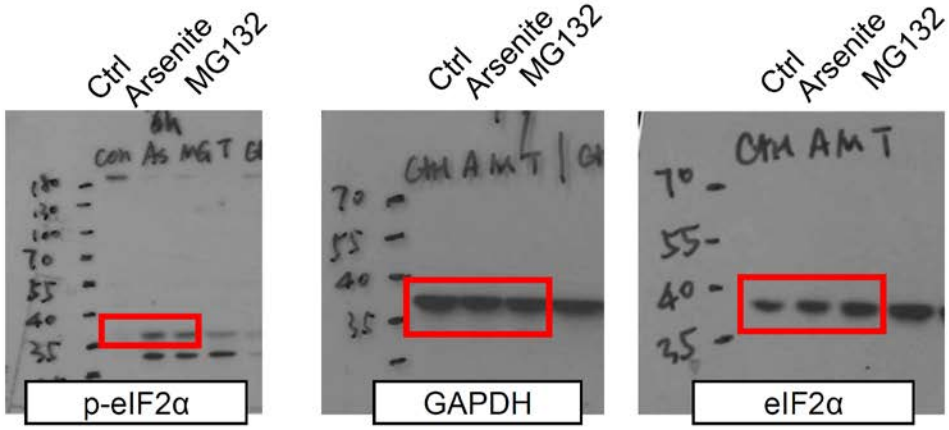


Figure 2d



Supplementary Figure 4 (cont'd): Uncropped Western Blots shown in main figures.

Figure 4c

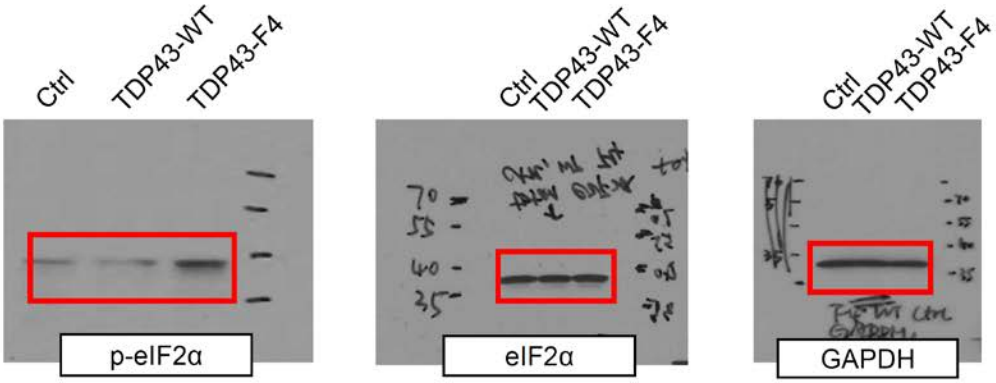
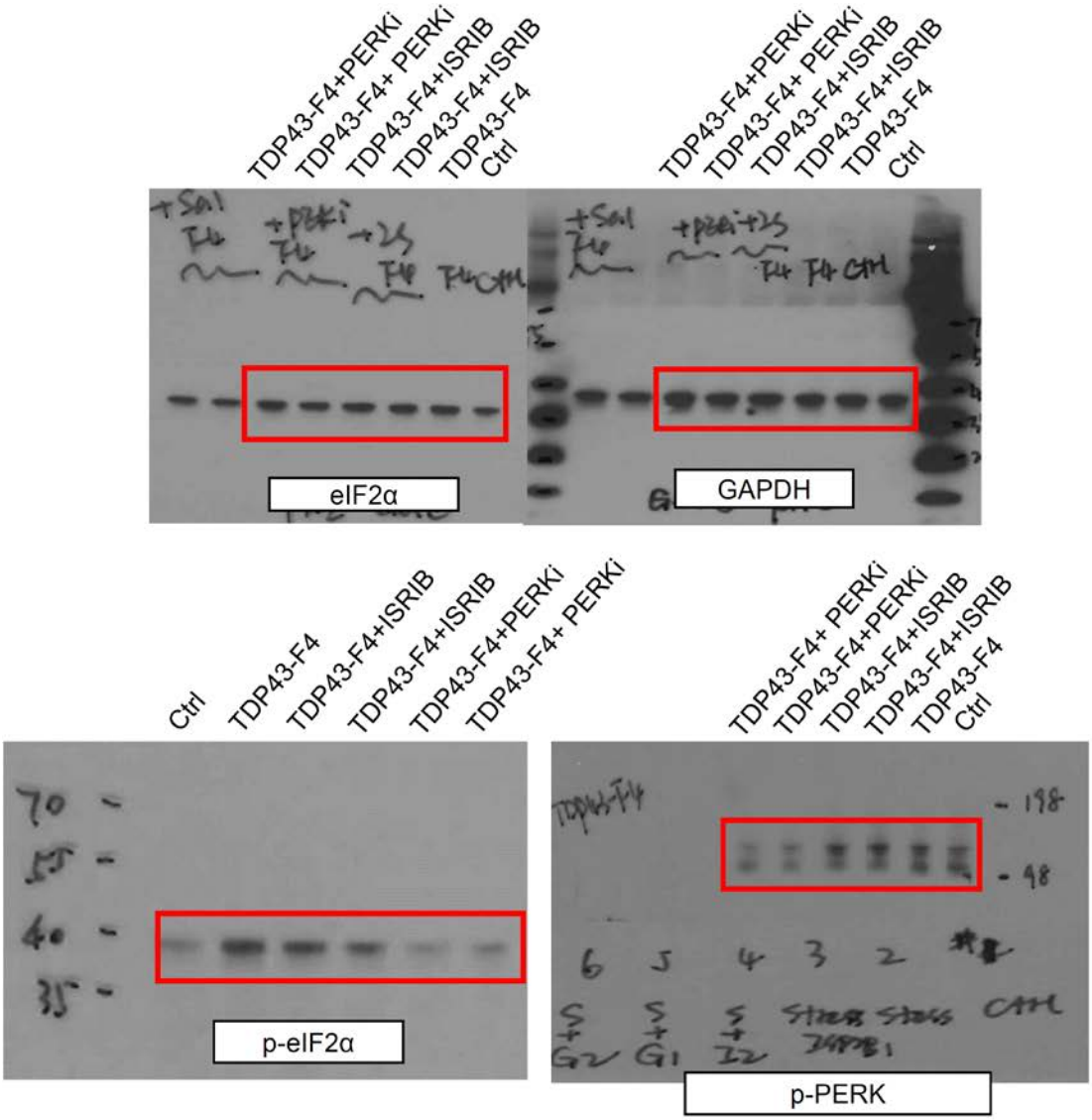


Figure 4e



Supplementary Figure 4 (cont'd): Uncropped Western Blots shown in main figures.

Figure 5c

Bicistronic splicing reporter

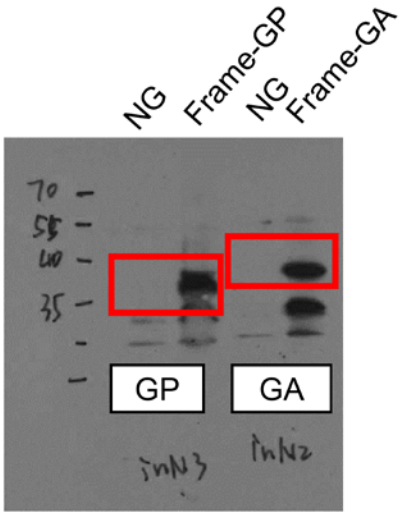


Figure 6a

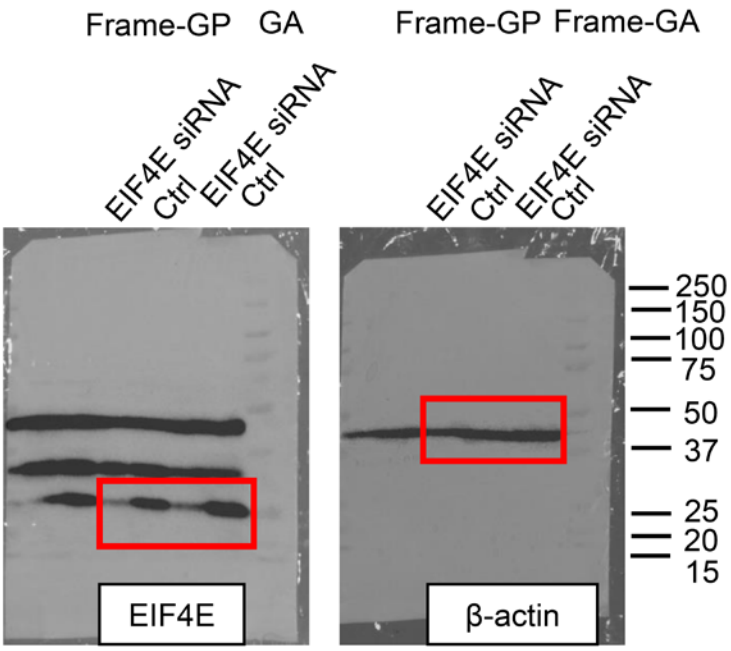
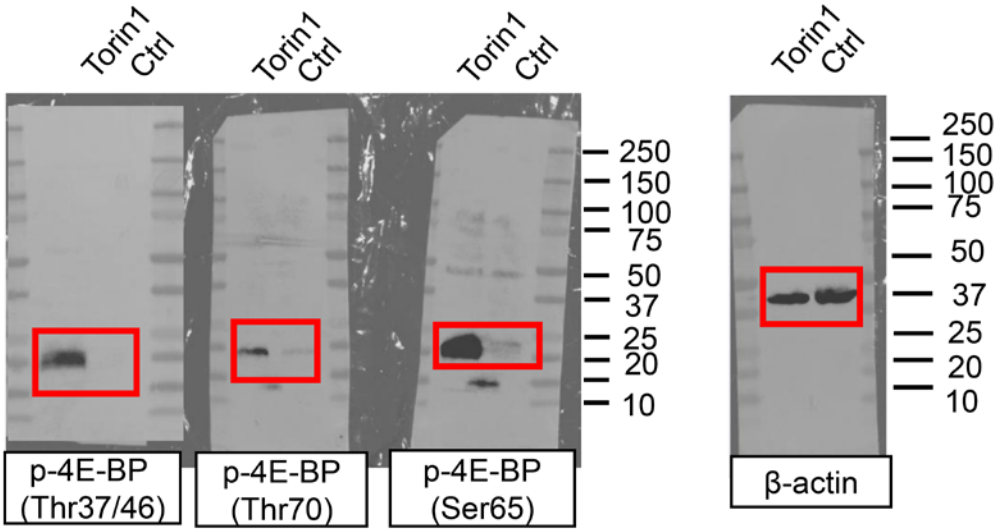


Figure 6c



Supplementary Figure 4 (cont'd): Uncropped Western Blots shown in main figures.

Figure S2c

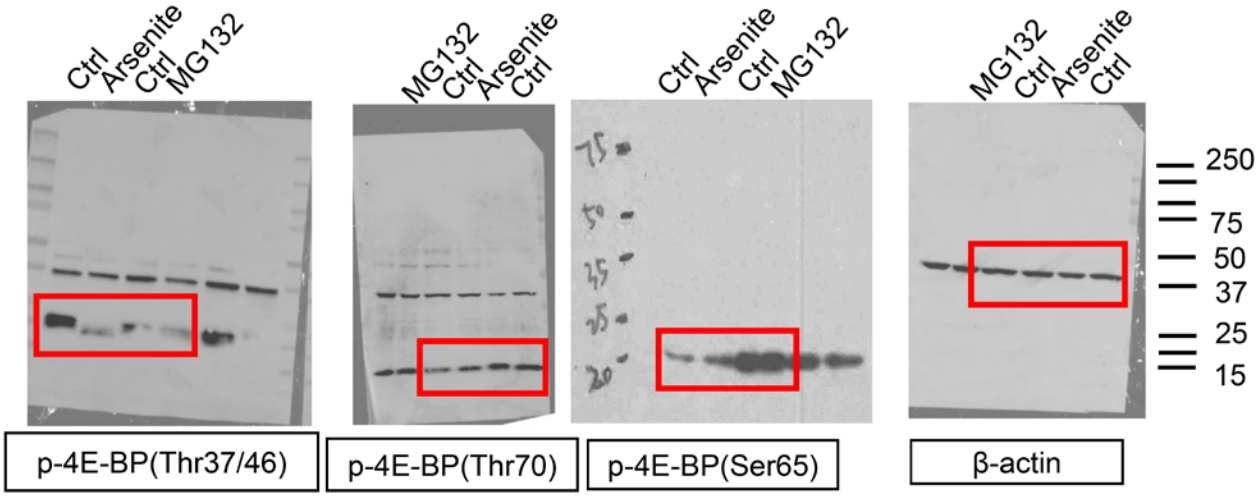
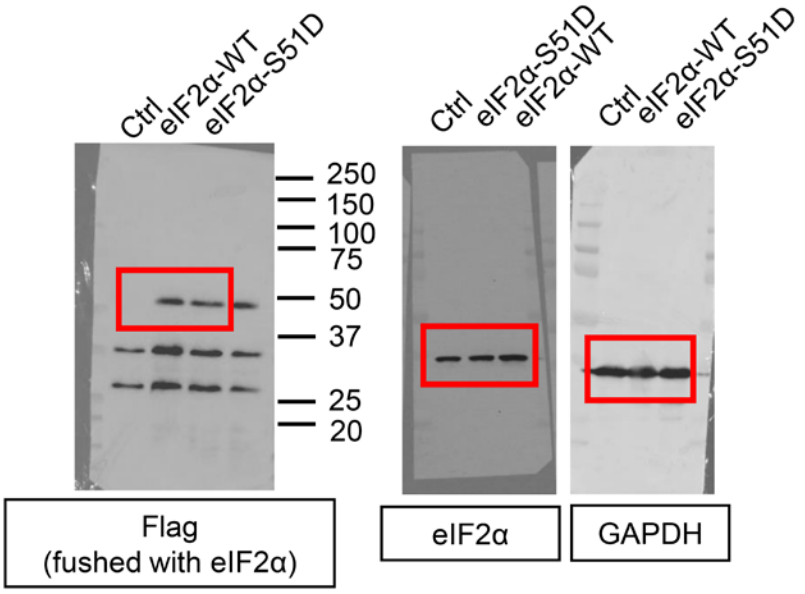


Figure S2d



Supplementary Figure 4: Uncropped Western Blots shown in main figures.

Supplementary Table 1: qPCR primer sequences.

NLuc	Forward	5'-GTCCGTAACTCCGATCCAAAG-3'
	Reverse	5'-TGCCATAGTGCAGGATCACCT-3'
FLuc	Forward	5'-GTGACTTCCCATTTGCCACC-3'
	Reverse	5'-TGATCTGGTTGCCGAAGATG-3'
GAPDH mRNA	Forward	5'-GAGTCAACGGATTTGGTCGT-3'
	Reverse	5'-TTGATTTTGGAGGGATCTCG-3'
MTRNR1	Forward	5'-CCCTGAAGCGCGTACACACC-3'
	Reverse	5'-GTCCAAGTGCACTTTCCAGT-3'
GAPDH pre-mRNA	Forward	5'-AAGGTGAAGGTCGGAGTCAAC-3'
	Reverse	5'-GCTGACCTTGAGCTCTCCTTG-3'
reporter spliced exon	Forward	5'-GAGGTGCGTCAAACAGCGAC-3'
	Reverse	5'-TTTGGCATCTTCCCTCGAGG-3'
reporter pre-mRNA	Forward	5'-GAGGTGCGTCAAACAGCGAC-3'
	Reverse	5'-AGAGCAAGTAGTGGGGAGAG-3'