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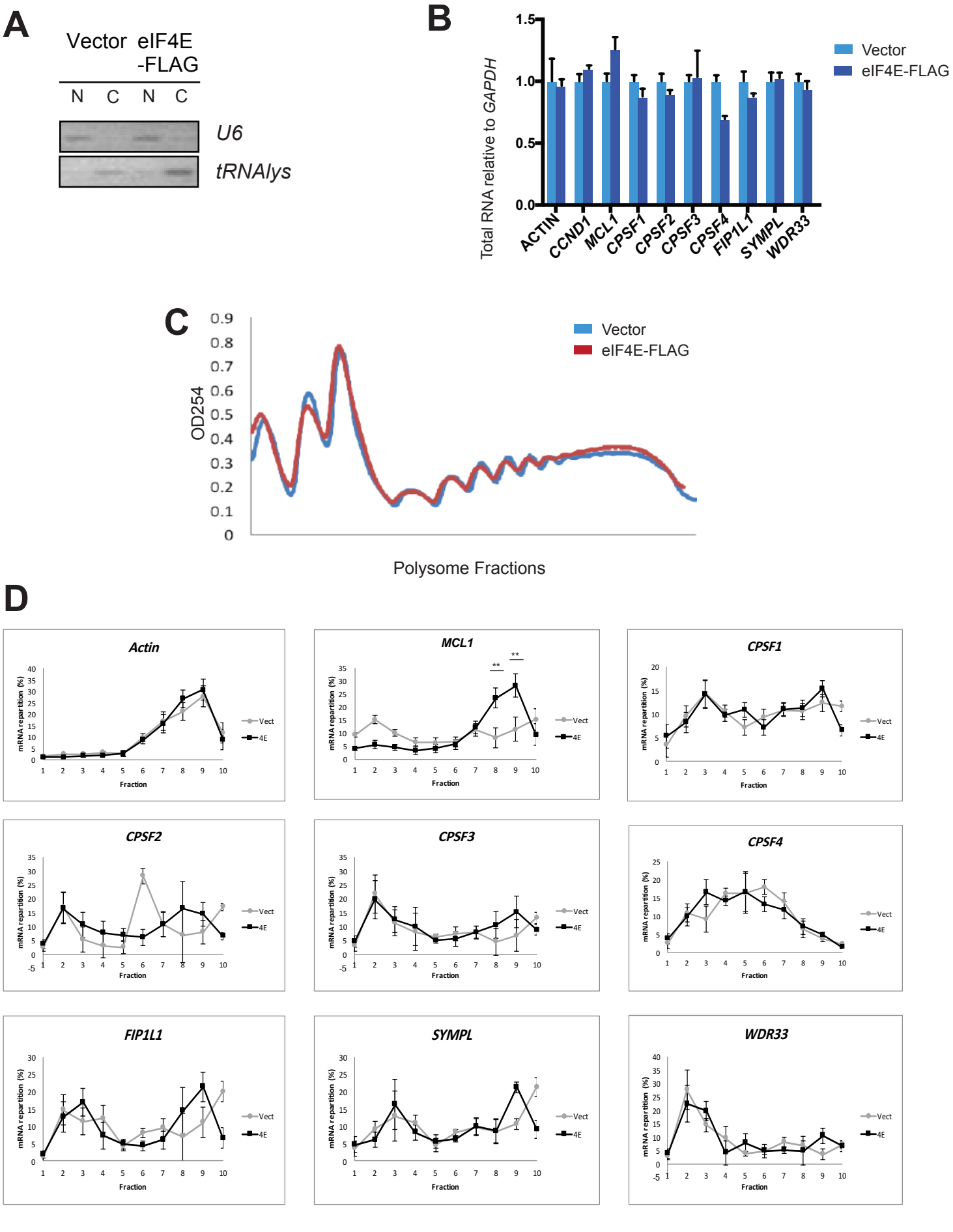
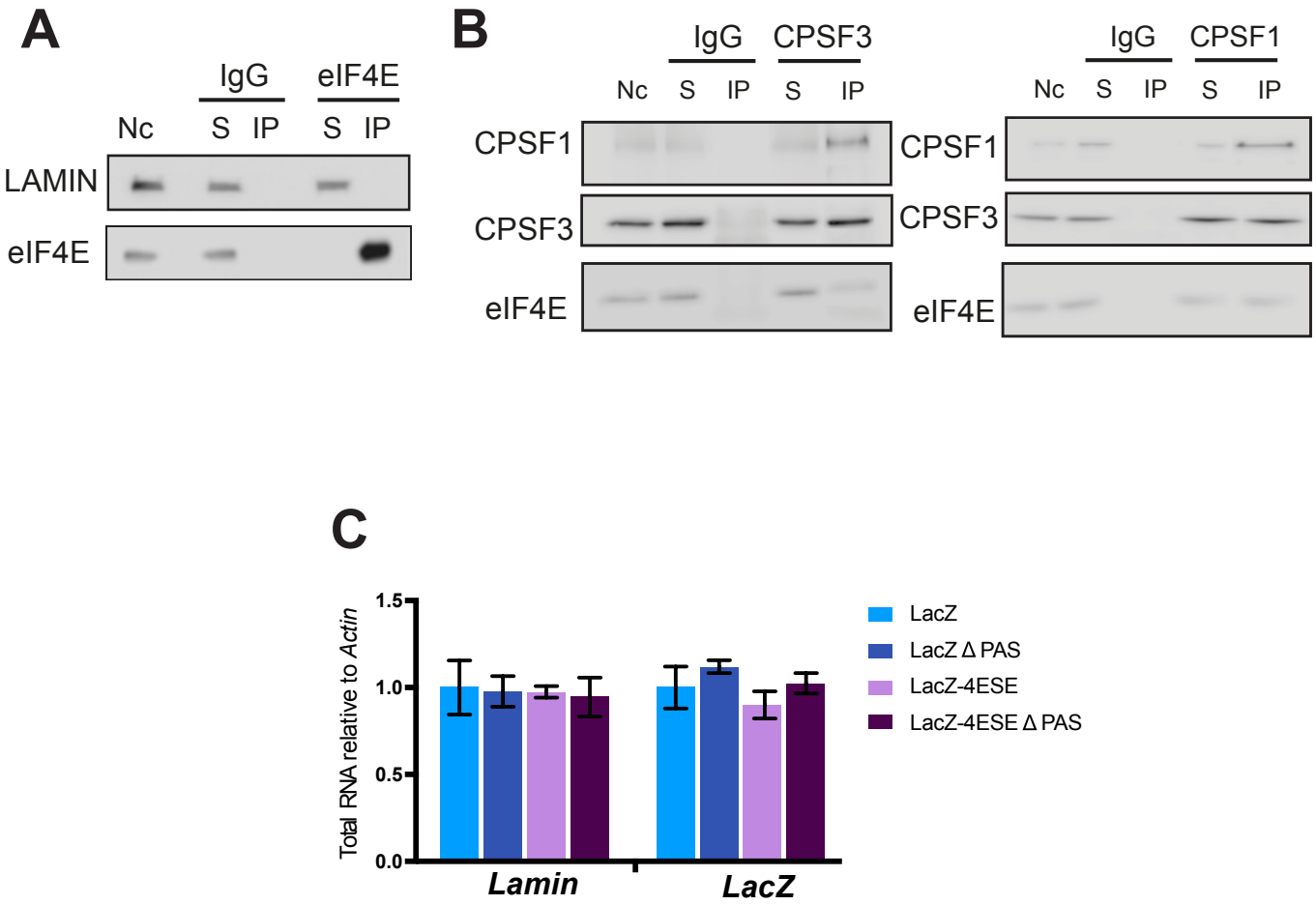
Supplemental Figure Legends and Table



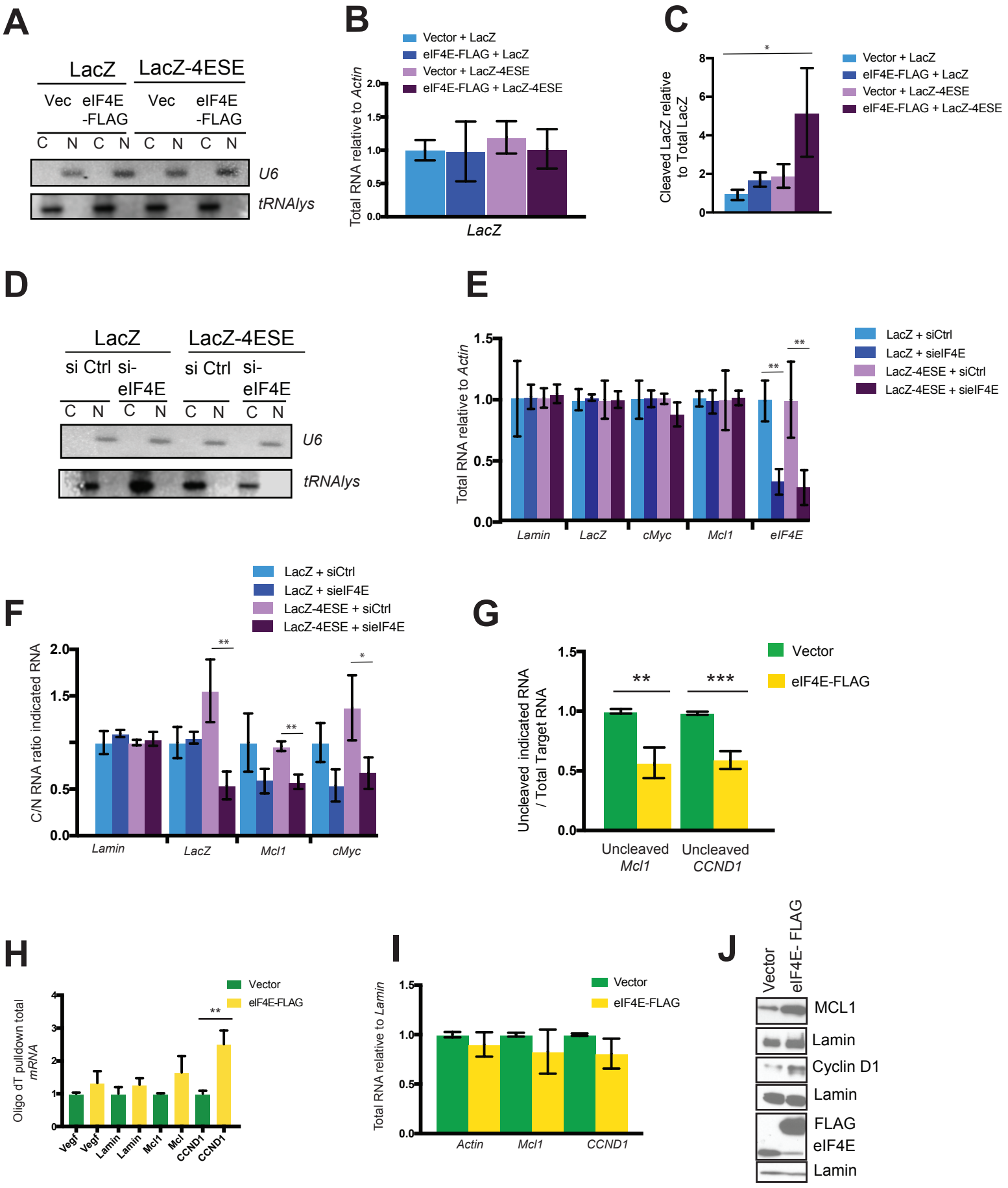
Supplemental Figure 1

Supplemental Figure 1. eIF4E does not modulate polysomal loading of CPA machinery related to Figure 1. **A.** Fractionation control for eIF4E-FLAG or vector controls for Figure 1. U6 is a marker of the nuclear fraction (N) and $tRNA^{lys}$ is a marker for the cytoplasmic fraction (C) as detected by semi- quantitative PCR. **B.** Total RNA levels for indicated RNA in vector or eIF4E-FLAG cells detected by RT-qPCR and normalized to *GAPDH*. **C.** Polysome profile (Fraction 1-10) is unchanged by eIF4E overexpression, as expected. OD_{254} across the profile. **D.** Polysome analysis for indicated RNAs across fractions. *MCL1,* a positive control for both export and translation efficiency, translation efficiency is increased. The remaining transcripts do not show a significant increase in eIF4E overexpressing cells. Means+/-standard deviation are shown. Experiments are from 4 biological replicates and RNAs detected by RT-qPCR normalized to Vector or eIF4E-FLAG input.



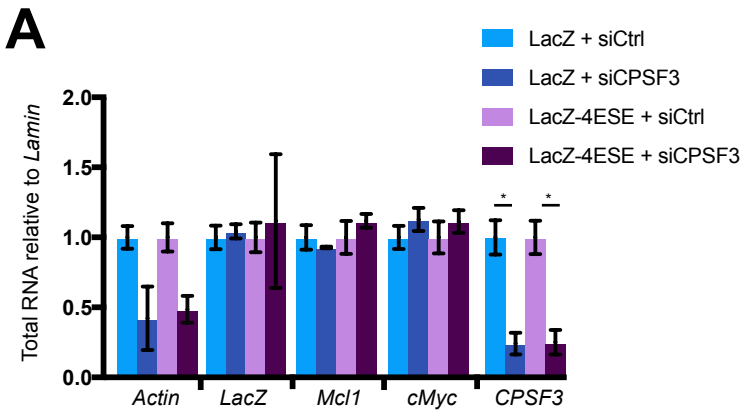
Supplemental Figure 2. eIF4E interacts with CPSF3 but not LAMIN related to Figure 2. A. eIF4E does not immunoprecipitate with negative control, LAMIN. Lysates used are the same as in Figure 2, but ran on a different gel. **B.** Endogenous eIF4E physically associates with CPSF3 and CPSF1 in U2Os cells. Nc, nuclear lysate, S, supernatant. **C.** Total RNA for indicated LacZ constructs in eIF4E-FLAG cells. *Lamin* RNA serves as a loading control. RNAs were detected by RT-qPCR, and values are means+/-standard deviations. Experiments were done in 3 biological replicates.

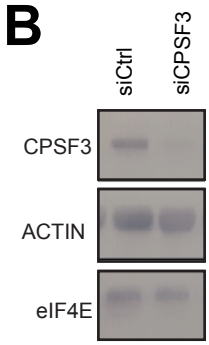
Supplemental Figure 2



Supplemental Figure 3

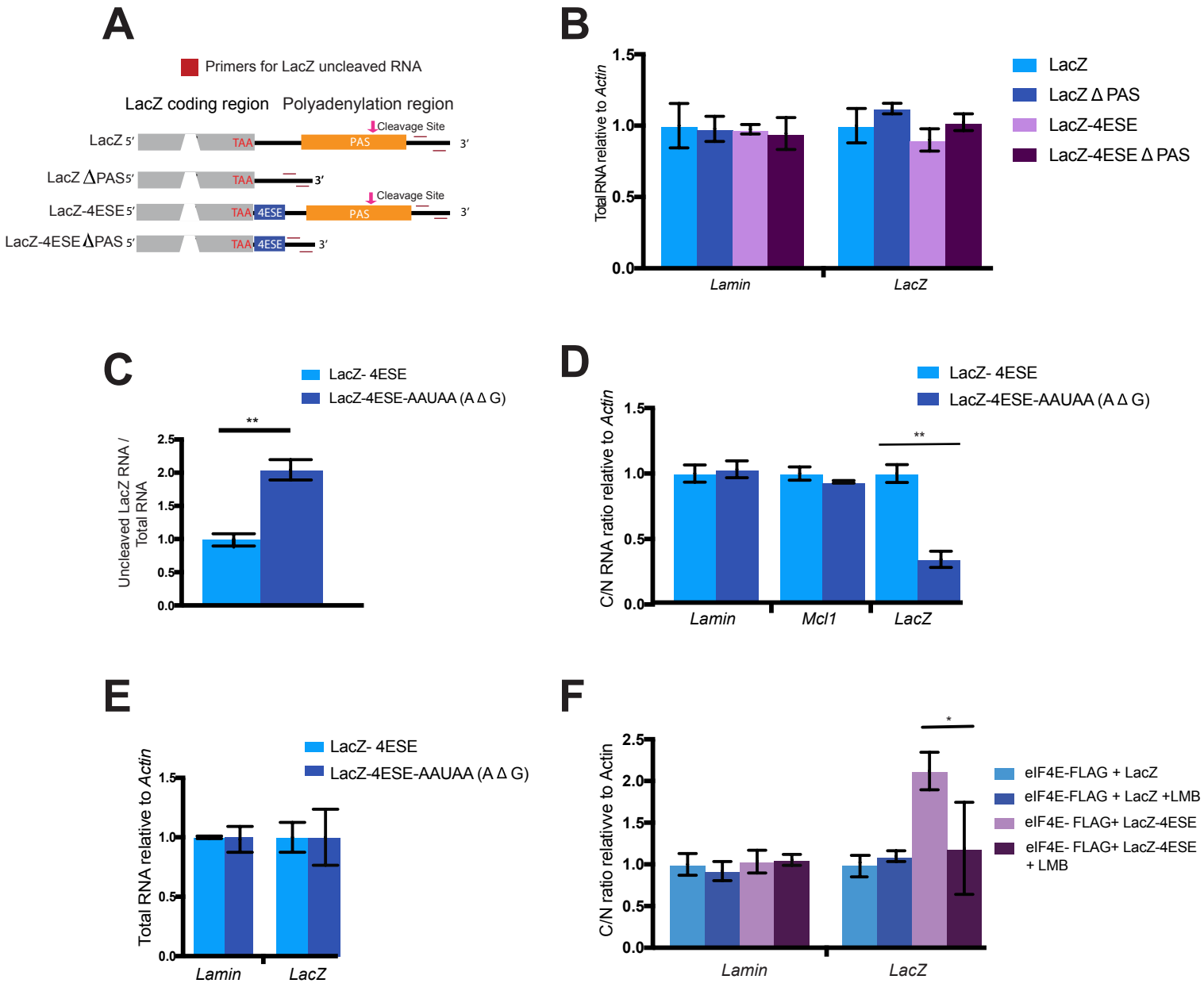
Supplemental Figure 3. **Endogenous RNA cleavage and controls related to Figure 3. A**. Fractionation controls for Figure 3 as described above**. B.** Total RNA levels for LacZ transcripts indicated from RT-qPCR. **C.** Cleavage assay using primers to detect cleaved and polyadenylated forms of LacZ. More cleaved RNA is observed in eIF4E overexpressing cells for the LacZ-4ESE construct. **D.** Fractionation controls for RNAi to eIF4E experiments in Figure 3. **E.** Total RNAs for LacZ constructs and on ctrl RNAs from Figure 3 from RT-qPCR assays. eIF4E RNA levels are the only significant change, whereby they were reduced by siRNA to eIF4E as expected. **F.** RNA export assay monitoring cytoplasmic (C) to nuclear (N) ratio on indicated RNAs as a function of si*eIF4E* relative to siCtrl using RT-qPCR. **G.** Uncleaved/total endogenous *MCL1* or *CCND1* (cyclin D1) transcripts measured in nuclear lysates from vector or eIF4E-FLAG U2Os cells. **H.** Oligo dT RNA pulldown from total cell lysates to assess fraction of RNA cleaved and RNA levels using exonic primers **(I). J.** Corresponding western blots for Mcl1 and cyclin D1. For RT-qPCR experiments, values are means +/-SD with P-values as follows: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$. Experiments were 5 (C), 3 (B, E, F, G, I) or 2 (H) biological replicates each carried out in triplicate. Representative fractionation controls are shown.





Supplemental Figure 4. Controls related to Figure 4. A. Analysis of total levels of RNAs indicated as a function of knockdown of *CPSF3* in eIF4E-FLAG U2Os cells. siCtrl indicates RNAi control. Means +/- standard deviations are shown. **B.** Western blot confirms knockdown of CPSF3, Representative blot from 3 biological replicates. ACTIN is shown as a loading control. RT-qPCR experiments were carried out in 3 biological replicates, each carried out in triplicate. Values are means +/-SD with P-values as follows: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

Supplemental Figure 4



Supplemental Figure 5. Controls related to Figure 5. A. Diagram of constructs used, not drawn to scale. **B.** Total RNA levels for *Lamin*, *LacZ* and *LacZ-4ESE* RNAs as a function of ΔPAS. **C.** Effects of point mutations in the PAS site (AAUAAA to AAUAAG) on cleavage. Uncleaved wildtype or mutant *LacZ-4ESE* were measured by RT-qPCR and elevated levels of uncleaved RNA indicated that mutation reduced PAS cleavage. **D.** RNA export assay as a function of PAS point mutation monitoring cytoplasmic (C) to nuclear (N) ratio relative to controls using RT-qPCR. **E.** Total RNA levels for the wildtype and point mutations show no differences by RT-qPCR. **F.** RNA export assay as a function of LMB treatment monitoring cytoplasmic (C) to nuclear (N) ratio relative to controls using RT-qPCR. Experiments were carried out in 3 biological replicates, each in triplicate. Values are means +/-SD with P-values as follows: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

Supplemental Figure 5

Name	Sequence
18S	FWD 5' CTGAGAAACGGCTACCACATC 3' RVS 5' GCCTCGAAAGAGTCCTGTCTTG 3'
LacZ uncleaved Fwd	5'CCCGTGCCTTCCTTGAC3'
LacZ uncleaved Rvs	5'ATGACACCTACTCAGACAATG3'
LacZ cleavage Rvs	5'TTTTTTTTTTTTGCGATGCAA3'
LacZ FWD O/H primers:	5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCC G TGCCTTCCTTGACCCTG 3'
LacZ RVS Uncleaved O/H primers:	5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGT AGAATGACACCTACTCAGACAA3'
LacZ RVS cleaved O/H primers:	5' GTCTCGTGGGCTCGGAGATGTGTA TAAGAGACAGTTTTTTTTTTTGCGATGCAATTTC 3'
LacZ total FWD:	5' ACAGCAAATGGGTCGGGATCTGTA3' (Invitrogen)
LacZ total RVS	RVS 5' TGTGCTGCAAGGCGATTAAGTTGG 3' (Invitrogen)
Actin B	FWD 5' TCTCTCTAAGGAGAATGGC 3' RVS 5'CATTTTTAAGGTGTGCACTT 3'
CCND1	FWD 5' CACTTCCTCTCCAAAATGCCA 3' RVS 5' CCTGGCGCAGGCTTGACTC 3'
CPSF1	FWD 5' CCTCACTGGCTCTTGGTGA 3' RVS 5' ATGGGGCATCATAGGACAGG 3'
CPSF2	FWD 5' GGCACTCCTAAATAATGTCAGTTACAA 3' RVS 5' TCAATTTATCACTCATCCATTCTACC 3'
CPSF3	FWD 5' ACTAGCCAAGGTTATGGGATTT 3' RVS 5' AGGTCGCAAGGAGAAAGTATG 3'
CPSF4	FWD 5' CGGCACACACGGAGAGTC 3' RVS 5' CTTTGCTGGAGGCTGTGTCT 3'
eIF4E	FWD 5' AGGAGGTTGCTAACCCAGAACACT 3' RVS 5' AAAGTGAGTAGTCACAGCCAGGCA3'

FIP1L1	FWD 5' CGCCTAGTGTCGGAGCTG 3' RVS 5' GGTCCTTTGCCAAATCACTG 3'
GAPDH	FWD 5' ACCACAGTCCATGCCATCAC 3' RVS 5' TCCACCACCCTGTTGCTGTA 3'
LacZ total	FWD 5' ACAGCAAATGGGTCGGGATCTGTA3' RVS 5' TGTGCTGCAAGGCGATTAAGTTGG 3'
LacZ △ PAS *uncleaved*	FWD 5' CCTGTAGCGGCGCATTAAG 3' RVS 5' GCGAGAAAGGAAGGGAAGAAAG 3'
LacZ AAUAAG	FWD: 5'CCACTGTCCTTTCCTAATAAGATGAGGAAATTGCAT CGCAT 3' RVS: 5'ATGCGATGCAATTTCCTCATCTTATTAGGAAAGGA CAGTGG 3'
Lamin	FWD 5' GCAAGACCCTTGACTCAGTAG3' RVS 5' GTCACCCTCCTTCTTGGTATTG3'
MCL1	FWD 5' ACTTCTCACTTCCGCTTCCTTCCA 3' RVS 5' TTTGAGGCCAAACATTGCCAGTCG3'
siRNA duplex _Luciferase	Sense: CACGUACGCGGAAUACUUCGAAAUG Antisense: CAUUUCGAAGUAUUCCGCGUACGUGUU
siRNA_duplex_*eIF4E* (Human)	Sense: CUGCGUCAAGCAAUCGAGAUUUGGG Antisense: CCCAAAUCUCGAUUGCUUGACGCAGUC
siRNA duplex _*CPSF3*	HSC.RNAI.N016207.12.1 (catalogue number) Sense: CCCUUCUAAUGAUAUGUAUGCAGAT Antisense: AUCUGCAUACAUAUCAUUAGAAGGGUU

Table S1. Oligonucleotides used in this study related to the STAR methods. Unless noted, these were obtained from Integrated DNA Technologies.